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The embryonic stem cell protein Oct4 may be a prognostic variable for death from ovarian cancer

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ABSTRACT

Ovarian cancer causes more deaths than any other cancer of the female reproductive system. The National Cancer Institute estimates that 21,990 women were diagnosed with and 15,460 women died from ovarian cancer in the U.S. last year. Likewise Cancer Research UK reports that approximately 6,600 women are diagnosed with and 4,400 women die from ovarian cancer in the UK each year. A better understanding of the molecular origins of ovarian cancer is urgently needed. A unique molecular trait of stem cells is the expression of the *oct4* gene, which is silenced following early embryonic development. Accumulating evidence suggests that *oct4* reactivation is a driving force in carcinogenesis. In this pilot study we examine Oct4 protein levels in tumor biopsy specimens from a random sample of patients with suspected ovarian cancer. We find that detectable Oct4 protein levels correlate strongly with the risk of death for patients with suspected ovarian cancer. The estimated hazard ratio of death for detectable Oct4 protein versus no detectable Oct4 protein was 18.9. A further Poisson regression analysis reveals that the hazard ratio increases significantly ($p = 0.0093$) with time since diagnosis. Our findings suggest that Oct4 protein is a prognostic variable for death from ovarian cancer, and also supports the concept that cancer stem cells are integral to the etiology of ovarian cancer.

KEYWORDS: cancer stem cell (CSC), oct4, oncogene, cellular reprogramming, induced pluripotent stem cell (iPS cell or iPSC), differentiation, prognostic variable

INTRODUCTION

In the western world, ovarian cancer is the most lethal gynecologic cancer, accounting for more deaths than endometrial and cervical cancer combined. Yet despite extensive research the long-term survival rate of ovarian cancer patients has not changed significantly over the past several decades, and remains low [1]. Current treatments for ovarian cancer are inefficient at targeting the cells that sustain tumor growth, and spread of metastatic disease due to late diagnosis frequently worsens the prognosis. A better understanding of the molecular origins of ovarian cancer is urgently needed to improve patient survival rates.

It has been suggested that tumors arise from cancer stem cells, whose capacity for self-renewal could sustain tumor growth, and that migrating or circulating cancer stem cells may enable the spread of metastatic disease [2-4]. Cancer stem cells have been isolated from various types of leukemia [5-8], and have also been identified in cancers of the central nervous system [9]. Albeit the isolation of cancer stem cells from solid

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tumors has so far proven elusive. For embryonic stem (ES) cells the ability to self-renew depends on the octamer-binding transcription factor 4 gene (*oct4*) [10]. The *oct4* gene (also called *pou5f1* or *otf3* or *oct3* or *oct3/4*) belongs to the *pit-oct-unc* (POU) homeodomain containing family of transcriptional regulators [11]. Its expression is normally tightly restricted to ES cells [12, 13]. Illegitimate *oct4* activation, resulting in neoplastic expression of the *oct4* gene, has thus emerged as a candidate biomarker for self-renewing cancer stem cells [14-16]. Here we examine Oct4 protein levels in tumor biopsy specimens from ovarian cancer patients.

MATERIALS AND METHODS

Collection of tumor biopsy specimens and histology

Surgical tumor biopsy specimens were collected from 20 random women with suspected ovarian cancer. One portion of each biopsy specimen was processed for histology (Table 1) according to a standardized protocol, and another portion of the biopsy specimen was subject to quantification of

Table 1. Histological classification and ICD-O-3 morphology codes for surgical tumor biopsy specimens.

Patient number	Age at time of biopsy (Years)	Histological classification	ICD-O-3[a] morphology code	Time between biopsy and death (Days)
1	47	Mucinous cystadenoma, NOS[b]	8470/0	Alive
2	62	N/A	N/A	Alive
3	54	Mucinous cystadenoma, borderline malignancy	8472/3	Alive
4	23	Teratocarcinoma	9081/3	Alive
5	71	Thecoma, NOS	8600/0	Alive
6	48	Mucinous cystadenocarcinoma, NOS	8470/3	Alive
7	36	Papillary serous cystadenocarcinoma	8460/3	Alive
8	52	Endometrioid carcinoma, NOS	8380/3	Alive
9	46	Serous cystadenoma, borderline malignancy	8442/1	Alive
10	72	Clear cell adenocarcinoma, NOS	8310/3	55
11	83	Papillary serous cystadenocarcinoma	8460/3	906
12	76	N/A	N/A	180
13	60	Carcinoma, undifferentiated, NOS	8020/3	1310
14	40	Carcinoma, undifferentiated, NOS	8020/3	1091
15	83	Mucinous cystadenocarcinoma, NOS	8470/3	3569
16	51	Adenocarcinoma, diffuse type	8145/3	951
17	70	Papillary serous cystadenocarcinoma	8460/3	652
18	69	Papillary serous cystadenocarcinoma	8460/3	943
19	62	Papillary serous cystadenocarcinoma	8460/3	658
20	64	Papillary serous cystadenocarcinoma	8460/3	902

[a]International Classification of Diseases for Oncology, Third Edition, Eds., A. Fritz, C. Percy, A. Jack, K. Shanmugaratnam, L. Sobin, D. M. Parkin and S. Whelan, Geneva, World Health Organization, 2000.

[b]Not Otherwise Specified.

Oct4 protein by Western blotting as described below.

Cell lines

Human fibroblasts and human SA121 ES cells were cultured under standard conditions and used as negative and positive controls for Oct4 protein respectively.

Western blotting

Quantification of Oct4 protein in tumor biopsy specimens and control cells was done by Western blotting. The analysis was repeated three times. For each sample 20 µl of homogenate was separated using standard SDS-PAGE and the protein content of the gel was transferred onto Immobilon-PSQ membrane (Millipore). The membrane was blocked in phosphate buffered saline containing 0.1% tween-20 (PBS-T) supplemented with 5% skimmed dried milk for 40 minutes at room temperature (RT), probed with rabbit anti-Oct4 (1:1000, H-134, sc-9081, Santa Cruz Biotechnology, Inc) and anti-β-actin (1:1000, C4, sc-47778, Santa Cruz Biotechnology,) overnight at +4 ºC, washed with PBS-T, incubated with alkaline phosphatase (AP) conjugated goat anti-rabbit IgM+IgG (H+L chain specific) (1:1000, Southern Biotech) and AP conjugated goat anti-mouse IgM+IgG+IgA (H+L chain specific) for 1 h at RT, washed with PBS-T, and developed in detection buffer (50 mM Tris pH 9.0, 5 mM $MgCl_2$, 66 % (v/v) nitro blue tetrazolium, NBT (50 mg/ml, Promega) and 33% (v/v) 5-bromo-4-chloro-3-indolyl phosphate, BCIP (50 mg/ml, Promega)).

Statistical analysis

Survival functions were determined by the Kaplan-Meier method. The death hazard function was estimated by a Poisson regression analysis, where the follow-up period of each patient was divided in intervals of the length 0.05 years. We studied the statistical interaction between the presence of Oct4 and time since diagnosis, by use of a Poisson model. All *p*-values are two-tailed.

RESULTS

Oct4 protein can be detected in tumor biopsy specimens from ovarian cancer patients

Tumor biopsy specimens from 20 random patients with histology-confirmed (Table 1) ovarian cancer were analyzed for the presence of Oct4 protein using Western blotting (Figure 1). Tumor biopsy specimens from eight patients were found to contain detectable levels of Oct4 protein whereas 12 patients lacked detectable levels of Oct4 protein.

The presence of Oct4 protein may be a strong prognostic variable for death from ovarian cancer

Median patient age of the cohort was 61 years (range 23-84 years) and total patient follow-up time of all patients together was 152.4 years. Nine patients are alive and may be considered

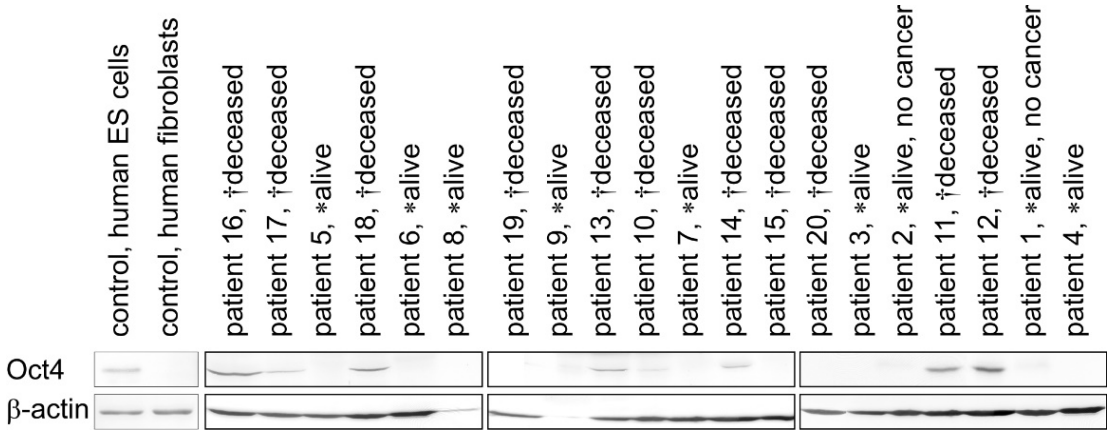

Figure 1. Oct4 protein can be detected in ovarian cancer patients. Western blotting reveals the presence of Oct4 protein in tumor biopsy specimens from ovarian cancer patients, and indicates that cancer stem cells may have a role in the etiology of ovarian cancer. The β-actin serves as gel loading control.

successfully treated whereas 11 patients have died from ovarian cancer since the biopsy (Table 1). Notably all of the patients whose tumor biopsy specimen contained detectable levels of Oct4 protein were deceased within four years (Figures 1 and 2). Survival functions were estimated by the Kaplan-Meier method and reveal that the probability of death is dramatically higher for patients whose tumor biopsy specimen contain detectable levels of Oct4 protein (Figure 2). The estimated hazard ratio (HR) of death for Oct4 versus no Oct4 was 18.9 (Table 2). A further Poisson regression analysis shows that the hazard ratio increased significantly ($p = 0.0093$) with time since diagnosis ($HR = \exp[0.374 + 1.184 \times \text{time}]$). Notably, at time ≥ 3.6 years HR exceeds 100.

Table 2. Estimation of the death hazard function by Poisson regression analysis.

Variable	**β**	**SE**	**HR (95% CI)**	***p*-value**
Constant	-6.1310	1.8260		
Time (years)	-0.0040	0.1383	1.00 (0.76 - 1.31)	0.9769
Current age	0.0372	0.0222	1.04 (0.99 - 1.08)	0.0930
Oct4 protein present (no = 0, yes = 1)	2.9386	1.0184	18.9 (2.57 - 139)	0.0039**

The hazard ratio (HR) was the ratio for a value of the current variable versus 1 unit lower value.
**$p < 0.01$.

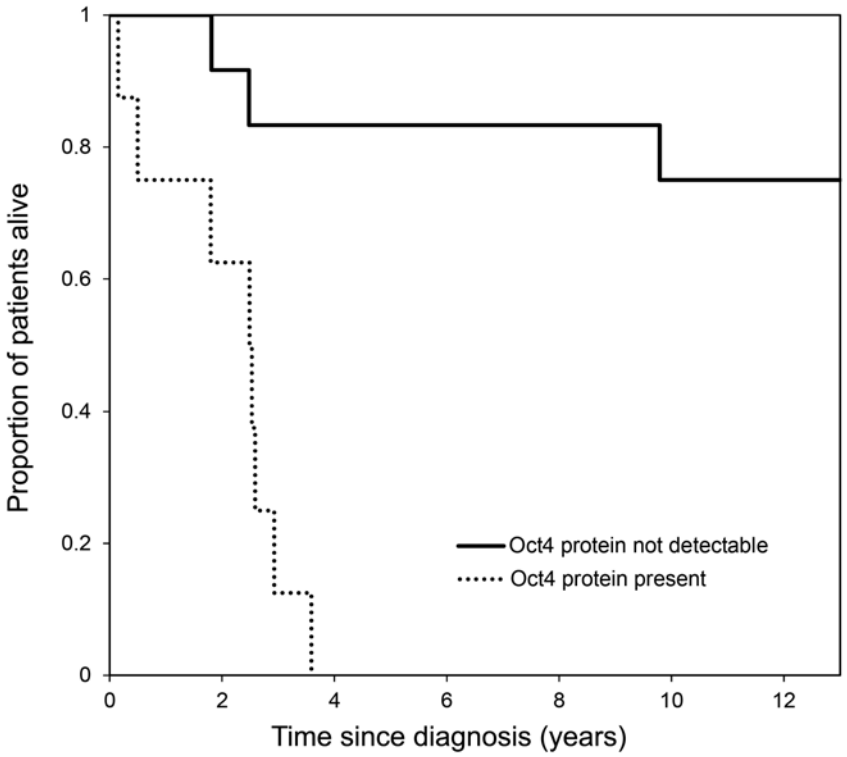

Figure 2. Oct4 protein may be a strong prognostic variable for death from ovarian cancer. A Kaplan-Meier plot reveals that the probability of death is dramatically higher for patients whose tumor biopsy specimens contain detectable levels of Oct4 protein.

DISCUSSION

A growing body of evidence supports the concept that cancer stem cells are important to the development and perpetuation of human cancers, and that eradication of the stem cell compartment of a tumor is necessary to achieve a stable long lasting remission or cure of the cancer [2, 7, 17, 18]. The *oct4* gene has recently been proposed to be a potent oncogene and a driving force in tumorigenesis [19], and recent findings have implicated *oct4* in the generation of tumor initiating cells [20]. The first ever paper showing a correlation between the expression of a gene involved in stem cell self-renewal and the prognosis for cancer patients was published in 2007, when Taubert *et al*. reported that Hiwi expression levels influence the risk of death for soft-tissue sarcoma patients [21]. Albeit to our knowledge the study presented here is the first report that links detectable Oct4 protein levels in tumor biopsy specimens to the risk of death for a specific type of cancer. Our study contains two facts of primary scientific interest. Firstly, the significant difference with respect to mortality between Oct4 positive patients and Oct4 negative patients ($p = 0.0039$) indicates that mortality is closely related to the presence of Oct4 protein per se. In this context we wish to stress that even though the ovarian cancer patient cohort in this pilot study is small, the reasonable evidence of a result with $p = 0.0039$ (or any other p-value) is not dependent on whether the number of patients is 20 or 20,000. Secondly, the extreme hazard ratio of death for Oct4 protein versus no Oct4 protein (HR = 18.9) suggests that Oct4 protein is a prognostic variable for death from ovarian cancer, and possibly also a target for intervention strategies.

For a continuous variable the predictive ability can be characterized by gradient of risk per one standard deviation. This quantity equals the hazard ratio when comparing two individuals, who differ one standard deviation with respect to the predictor. For example the gradient of risk of hip fracture depending on bone mineral density (BMD) measured at the hip is 2.6 [22]. The predictive ability of a zero-one variable can be compared to a normally distributed variable X by determination of a cutoff C and, a coefficient β, so the proportion of those above C equals the proportion of those assuming the value 1 on the zero-one variable and the probability of event for those above C divided by the probability of event for those below C equals the ratio of those with the value 1 of the zero-one variable versus those with the value 0. The ratio equals the quotient between two conditional expected values $E[\exp(\alpha+\beta\cdot X)|X>C]/E[\exp(\alpha+\beta\cdot X)|X\leq C]$. We calculated that detectable Oct4 corresponds to a normally distributed variable with a gradient of risk equal to 5.5, which is very high.

CONCLUSION

In summary, our results suggest that the presence of Oct4 protein in tumor tissue may be a strong prognostic variable for death from ovarian cancer, and also indicate that cancer stem cells could be integral to the etiology of ovarian cancer. We hope these findings will stimulate further research aiming to elucidate the molecular origins of ovarian cancer.

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CONFLICT OF INTEREST STATEMENT

None of the authors has a potential conflict of interest to declare.

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