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Variant HNF1 Modulates Epithelial Plasticity of Normal and Transformed Ovary Cells[1]

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Abstract

Ovarian carcinoma arises from the ovarian surface epithelium, which undergoes phenotypic changes characteristic of müllerian epithelium during the first stages of tumorigenesis. The variant isoform of the hepatocyte nuclear factor 1 (vHNF1) is a transcription factor involved in the development of tissues derived from the müllerian duct. Here, we show that vHNF1 knockdown in two ovarian carcinoma cell lines, SKOV3 and IGROV1, leads to reduced E-cadherin (E-cadh) expression and decreased proliferation rate. Accordingly, SKOV3 cells ectopically expressing a dominant-negative (DN) vHNF1 mutant undergo an epithelial-mesenchymal–like transition, acquiring a spindle-like morphology, loss of E-cadh, and disrupted cell-cell contacts. Gene expression profiling of DNvHNF1 cells on the basis of a newly compiled list of epithelial-mesenchymal transition–related genes revealed a correlation between vHNF1 loss-of-function and acquisition of the mesenchymal phenotype. Indeed, phenotypic changes were associated with increased Slug transcription and functionality. Accordingly, vHNF1-transfected immortalized ovarian surface epithelial cells showed down-regulation of Snail and Slug transcripts. In DNvHNF1-transfected SKOV3 cells, growth rate decreased, and in vHNF1-transfected immortalized ovarian surface epithelial cells, growth rate increased. By immunohistochemistry, we found a strong association of vHNF1 with E-cadh in clear cell and in a subset of serous carcinomas, data that could potentially contribute in distinguishing different types of ovarian tumors. Our results may help in understanding the biology of ovarian carcinoma, identifying early detection markers, and opening potential avenues for therapeutic intervention.

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Introduction

The pathophysiology of epithelial ovarian cancers (EOCs) remains poorly defined. One widely supported hypothesis is that they are derived from inclusion cysts. These cysts originate from the ovarian surface epithelium (OSE), which is the monolayer of cells covering the ovaries [1,2]. Ovarian surface epithelium cells appear as a simple epithelium with some characteristics typical of mesenchymal cells. Ovarian surface epithelium cells remain plastic in short-term culture, expressing vimentin together with cytokeratins 8 and 18. Conversely, invaginations and inclusion cysts have properties characteristic of müllerian epithelium, including expression of the specific epithelial marker E-cadherin (cadh) at the cell-cell junctions. After transformation,

Abbreviations: Ab, antibody; cadh, cadherin; ctn, catenin; DN, dominant-negative; EMT, epithelial-mesenchymal transition; EOC, epithelial ovarian carcinoma; FR, folate receptor; IHC, immunohistochemistry; LMP, low malignant potential; MET, mesenchymal-epithelial transition; NE, nuclear extract; OSE, ovarian surface epithelium; vHNF1, variant hepatocyte nuclear factor; wt, wild type

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EOC cells can coexpress E-cadh and the mesenchymal marker vimentin as well as epithelial cytokeratins [3]. Unlike the tumor suppressor function of E-cadh in breast, prostate, and colon carcinomas [4,5], expression of E-cadh in ovarian epithelium seems to be associated with the development of EOCs [6]. Nonetheless, the mechanism of E-cadh–associated malignant OSE transformation is controversial [7,8]. In some advanced-stage EOCs, the so-called mesenchymal-epithelial transition (MET), which occurs during the first stages of transformation, is followed by an epithelial-mesenchymal transition (EMT) with loss of E-cadh expression [9].

Epithelial-mesenchymal transition is required for morphogenesis during embryonic development but has also been implicated in the acquisition of invasiveness by end-stage tumors [10–12]. This conversion results in loss of expression of adhesion molecules, such as E-cadh, ZO-1, and occludin, with consequent loss of cell-cell contacts and extensive remodeling of the cytoskeleton. Loss of E-cadh during development and cancer progression in tumors, other than EOCs, is mainly caused by transcriptional repression resulting from interaction of regulators with specific E-boxes in the proximal promoter of *Cdh1*, the gene encoding E-cadh [13]. Most prominent in this respect are the Snail-related zinc-finger transcription factors Snail and Slug.

The variant isoform of the transcription factor HNF-1 (vHNF1) activates transcription on homodimerization or heterodimerization with its companion protein HNF1α [14]. A role for HNF1 proteins in tumors has not yet been defined. For HNF1α, a biallelic inactivation of the relevant gene has been found in 50% of human liver adenomas [15], and somatic mutations were observed in 11% of endometrial carcinomas but not in breast and ovarian carcinomas [16]. Regarding vHNF1, the complete inactivation by germ line mutation of *TCF2*, the gene encoding for vHNF1, seemed to be associated to renal cell carcinoma [17] hypothesizing a tumor suppressor function. More recently, two variants within TCF2 have been found to be associated to prostate cancer risk [18]. vHNF1 is involved in the development of tissues organized in tubules, such as the pancreatic exocrine ducts and the kidney tubules [19,20], and in müllerian duct–derived tissues [21]. The transcription of the *FR* gene, which encodes the folate receptor (FR) α, is strongly activated in EOCs. We recently showed that the *FR* gene is regulated by vHNF1 [22], which is expressed in ovarian tumor specimens but not in OSE cells or in specimens obtained from tumors of other oncotypes.

Here, we addressed the potential role of vHNF1 in the MET-like taking place during ovarian cell transformation. We used *in vitro* approaches to negatively or positively affect vHNF1 expression and/or functionality in ovarian normal and transformed cells. We found that vHNF1 expression and functionality are directly correlated with epithelial differentiation, positively associated with growth potential, and inversely correlated with expression and functionality of E-box–binding transcriptional repressors. Immunohistochemical analysis of normal and transformed ovarian tissues showed that vHNF1 is not expressed in OSE cells but is expressed in 33% of E-cadh–expressing EOCs independently of tumor grading. The overall results demonstrate that vHNF1 is a new player in the epithelial differentiation of a subset of normal and transformed ovary cells.

Materials and Methods

Cell Culture

The ovarian carcinoma cell lines IGROV1 and SKOV3 (American Type Culture Collection, Manassas,VA) were maintained in RPMI 1640 medium (Sigma, St. Louis, MO) supplemented with 10% FCS (Sigma) and 2 mM L-glutamine. hTERT-IOSE (hereafter designated IOSE), obtained as described [23], were maintained in 199-MCDB105 medium (Sigma) supplemented with 15% FCS, 2 mmol L-glutamine, 200 μg/ml G418, and 50 μg/ml hygromycin.

Reagents and Antibodies

Triton X-100 (TX-100) and MES were from Sigma-Aldrich Fine Chemicals (St. Louis, MO); geneticin sulfate (G418) was from Gibco BRL (Paisley, Scotland). The following primary antibodies (Abs) were used at the dilution recommended by the manufacturer: anti-vHNF1 (goat), anti-HNF1 (rabbit), anti–ZO-1, and anti–occludin 1 (Santa Cruz Biotechnology, Santa Cruz, CA); anti–E-cadh mAb (mouse; Transduction Laboratories, BD Biosciences Pharmingen, Palo Alto, CA); anti-S100A4 (rabbit; DakoCytomation, Glostrup, Denmark). Horseradish peroxidase–labeled secondary Abs were from Amersham Bioscience–GE Healthcare (Piscataway, NJ). Secondary fluorochrome–conjugated Alexa Fluor 488 (green) was from Molecular Probes (Eugene, OR).

Small interfering RNA Treatment

IGROV1 and SKOV3 cells (5×10^5) were seeded in 24-well plates and transfected 24 hours later with 80 pmol/ml of small interfering RNA (siRNA) duplex against vHNF1 mRNA (SmartPool; Dharmacon, Lafayette, CO) or Luciferase siRNA as control (Quiagen-Xeragon, Germantown, MD). siRNA transfection was performed by using Lipofectamine 2000 (Invitrogen, Paisley, UK) according to the manufacturer's protocol. Cells were harvested 48 hours later and analyzed for RNA and protein expression by quantitative reverse transcription–polymerase chain reaction (RT-PCR) and Western blot analysis, respectively.

Construction of DNvHNF1 and vHNF1 Expression Vectors

vHNF1 cDNA was obtained from the vector RSV-LFB3 (kindly provided by C. Toniatti, IRBM, Merck Research Laboratories, Pomezia, Italy). Dominant-negative vHNF1 (DNvHNF1; nt 1-729 of the open reading frame) was obtained by standard PCR with sense and antisense primers containing *Hin*dIII and *Xba*I restriction sites, respectively (sense, 5′-AGGAGGTCTAGAATGGTGTCCAAGCTCACG-3′; antisense, 5′-AAGGGAAGCTTTCACCAGGCTTGTAGAGG-3′). The purified fragment was inserted into the *Hin*dIII and *Xba*I sites of the expression vector pcDNAIneo (Invitrogen). For the expression vector encoding vHNF1, the vHNF1 open reading frame was inserted into the *Hin*dIII and *Xba*I restriction sites of the pcDNA3.1/Hygro vector (Invitrogen). Before transfection, both vHNF1-pcDNA3.1/Hygro and DNvHNF1-pcDNAIneo were verified by sequencing.

Quantitative Real-time RT-PCR

Total RNA was isolated with the RNeasy Total RNA kit (Quiagen, Hilden, Germany) according to the manufacturer's instructions. One microgram of total RNA was reverse-transcribed using the ABI High Capacity cDNA Archiving Kit (Applied Biosystem, Foster City, CA). Three replicates were run for each gene in each sample in a 96-well format plate. The probes and primer sets were the following Assays on Demand: Ref Hs00170423_m1 for *Cdh1*, Hs00195591_m1 for *Snail*, Hs00161904_m1 for *Slug*, HS00170182_mi for *PLAU*, and Hs00277509_m1 for *FN* (Applied Biosystems). *GADPH* mRNA levels were used as a control for the RNA extraction and RT experiments. Data were analyzed with the Sequence Detector v1.9 software. Relative