Characteristics and differentiated mechanism of vascular endothelial cells-like derived from epithelial ovarian cancer cells induced by hypoxia

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Abstract. A few highly aggressive and malignant tumor cells could acquire identities by turning on genes expressed by endothelial cells and recruit blood vessels to sustain tumor growth. Hypoxia was reported recently to play an essential role in these events. These 'plastic' tumor-cell phenotypes and the exact mechanism driving transendothelial differentiation by hypoxia-inducible factor (HIF)-1α is unclear. In this study, epithelial ovarian carcinoma cells were exposed to hypoxia and the tumor cells were transformed into endothelial cells-like (ECs-like). Typical endothelial features such as cell markers and uptaking of acetylated low density lipoprotein were identified constantly. Small interference RNA was used to block the expression of HIF-1α. Analysis revealed that hypoxia promotes transendothelial differentiation through stimulating HIF-1-dependent transcriptional expression of vascular endothelial growth factor (VEGF), VEGF receptor-2 (Flk-1) and P53, and through decreasing HIF-1-independent transcriptional expression of Cyclin D1. These results demonstrate that ECs-like derived from epithelial ovarian cancer cells are similar to endothelial progenitor cells rather than endothelial cells. HIF-1α is crucial but not unique in alternation of tumor cells towards ECs-like.

Introduction

Tumor angiogenesis is of critical importance in the development and metastatic spread of tumors (1). It has been taken for granted that all intratumoral vascular channels are formed and lined by endothelial cells (ECs) (2). A novel microcirculation was recently described in uveal melanomas by which aggressive tumor cells generate non-EC-lined channels delimited by extracellular matrix and is independent of angiogenesis. 'Vasculogenic mimicry' was a name assigned to this process (3). There was preliminary evidence that this phenomenon occurs in some highly aggressive tumors including prostate tumor, lung carcinoma, breast cancer and glioblastoma cancer (4-6). Tumor cells are provided with direct access to the bloodstream by the new type of blood vessels and tend to spread aggressively. Some current antiangiogenesis efforts might have overlooked the blood vessels made by the actual cancer cells. Patients with vasculogenic mimicry were very likely to develop metastases with shorter life expectancy (7). The finding calls for reappraisal of the current assumption that endothelial cell-mediated angiogenesis is the only mechanism underlying or responsible for tumor growth and metastasis and poses challenges to antiangiogenesis strategies of cancer treatment. Microarray analysis found that cancer cells acquired identities by activating genes expressed by key ECs (8). These observations prompt further investigation into the potential relevance of a 'plastic' tumor-cell phenotype. Identifying characteristics of these channel-lined cells will provide new perspectives to combat the tumor vessel formation.

Hypoxia, one of the essential features of tumor microenvironment, is associated with neoplastic malignant progression and poor prognosis. Hypoxia can induce tumor angiogenesis, drive clonal selection, change expression of suppressor genes or oncogenes, contribute to resistance to chemo- and radio-therapies as well as lead to differentiation (9-16). Jögi *et al* demonstrated that human neuroblastoma cells grown under hypoxic conditions lose their neuronal and develop a neural crest-like phenotype (17). Hypoxia also induces dedifferentiation and drives promotion of the aggressive phenotype in ductal breast cancer (18). Hypoxia is known to be a critical mediator of tumor angiogenesis through hypoxia-inducible factor (HIF)-1α (9). Studies have recently shown that hypoxia is of great importance in the development of vasculogenic mimicry (19-21), but the exact mechanism by which HIF-1α acts in differentiation of aggressive tumor cells towards endothelial cells-like (ECs-like) that are involved in vasculogenic mimicry remains to be elucidated.

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Ovarian carcinoma mainly consisting of epithelial cancer is the leading cause of death from gynecologic cancers. Vasculogenic mimicry has been described in ovarian cancer previously (22,23). Based on the assumption that the microenvironment has powerful influence in determination of cell differentiation (24,25), we created a supportive environment with low oxygen tension on appropriate scaffolds to direct differentiation of epithelial ovarian cancer cells SKOV-3 and ES-2 into ECs-like. Typical endothelial features such as cell markers, microstructures and uptaking of acetylated low density lipoprotein (acLDL) were identified by immunofluorescence, RT-PCR and electron microscopy. Small interference RNA (siRNA) was used to block the expression of HIF-1α. After laser capture microdissection, the role of HIF-1α in tumor cell transendothelial differentiation was analyzed by quantitative real-time RT-PCR. The present study suggested that the differentiated ECs-like associated with tumor stem cells possess some typical EC characteristics. HIF-1α-modulated differentiation is an important mechanism in transendothelial differentiation of ovarian tumor cells.

Materials and methods

Three-dimensional cultures and hypoxic treatment. Human epithelial ovarian carcinoma cell lines SKOV-3 and ES-2 were purchased from American Type Culture Collection (ATCC, Manassas, VA), and were maintained in McCoy's 5a. Thirty microliters of Matrigel (B&D, Bedford, MA) were dropped onto each glass coverslip in a 12-well culture plate and polymerized for 1 h at room temperature, then 30 min at 37˚C in a humidified 5% CO_2 incubator, as described previously (22). Tumor cells ($1x10^4$) were seeded onto the three-dimensional gel. The medium with 15% FBS was changed every 36 h. Hypoxic condition was created by flushing 5% CO_2 and 95% N_2 through a modified chamber (Mitsubishi, Japan), until O_2 concentration was reduced to 1%, measured with a Mini oxygen meter. The culture system was then sealed and incubated at 37˚C (14).

Small interference RNA. To demonstrate the specificity of siRNA against HIF-1α, the following oligonucleotides were used: position 127, 5'-CAGTTGCCACTTCCACATA-3'. Oligonucleotides were synthesized and ligated into pRNAT-U6.1/Neo vector by Kanchen Biologic Technology Co. (Shanghai, P.R. China) according to the manufacturer's directions. A total of $2x10^5$ cells were seeded into each well of a 24-well plate without gentamicin. On the following day (when the cells were 85-90% confluent), the culture medium was aspirated and cells were transfected with 0.8 μg of the silencing plasmid pRNAT-U6.1/Neo and 2 μl Lipofectamine 2000 (Invitrogen, Carlsbad, CA). As control, cells were exposed to Lipofectamine 2000 without siRNA encoding plasmids, and oligonucleotides which do not have any target region in human genes served as negative control. Twenty-four hours after transfection, the resultant transfection efficiency using constructs expressing GFP assessed by fluorescence microscopy was ~65% (ES-2) and ~80% (SKOV-3) respectively. Stable transfected cells were selected by 0.4 mg/ml (SKOV-3) or 0.5 mg/ml (ES-2) G418 (Merck, Darmstadt, Germany) after approximately 2 weeks. The efficiency of HIF-1α silencing was analyzed by Western blotting.

Light and electron microscopy. Any extra-cellularly derived structures were directly examined by light microscopy (Leica, Wetzlar, Germany), and photographed with digital camera. For scanning electron microscopy and transmission electron microscopy, tissue cultures were fixed in cold 2.5% glutaraldehyde in 0.1 mol/l of sodium cacodylate buffer and post-fixed in osmium. Specimens were then either embedded, sectioned, and stained by routine procedures for Philips CM 120 transmission electron microscopy, or critically point-dried, and sputter-coated with gold for Hitachi S-520 scanning electron microscopy, as previously described (26). Semithin sections were studied by light microscopy and ECs-like were spotted when tissues were sectioned for transmission electron microscopy.

Immunofluorescence assay. After the cells were allowed to grow channels on glass coverslips, cultures were washed with PBS and fixed with 3% paraformaldehyde at 4˚C for 5 min, subsequently permeabilized with 1% Triton X-100 for 10 min and washed with PBS. Cultures were incubated with primary monoclonal antibodies (mouse anti-human B blood type in SKOV-3 cultures, Zymed, South San Francisco, CA; mouse anti-human P glycoprotein in ES-2 cultures, Santa Cruz, Santa Cruz, CA, respectively) and washed with PBS, then incubated with TRITC-labeled goat anti-mouse secondary antibody (Friendship Biotechnology, Shanghai, P.R. China), respectively, for 90 min at 37˚C in humidified blind incubator. After completing the first staining, cultures were incubated with primary polyclonal antibodies including rabbit anti-human vWF antibody (NeoMarkers, Fremont, CA), rabbit anti-human CD34 antibody (Santa Cruz), rabbit anti-human AC133 antibody (Novus Biologicals, Littleton, CO), and rabbit anti-human Flk-1 antibody (Neomarkers) respectively, and then treated with FITC-labeled mouse anti-rabbit secondary antibody (Friendship) to complete the second staining according to the first staining process described above. The images were obtained by fluorescence microscopy (Leica). All negative controls were processed by omitting the primary antibody.

Uptake of acLDL. Twenty μg/ml DiL-acLDL (Molecular Probes, Eugene, OR) was added to media, and incubated with cultured cells on gel for 4 h at 37˚C in a humidified incubator on the 8th day, and then washed twice with PBS. Human umbilical vein endothelial cells and SKOV-3 or ES-2 cells were used as a positive and negative reference, respectively. The images were obtained using a fluorescent microscope.

Laser capture microdissection. Fifteen mocroliters of Matrigel were mounted on ethylene vinyl acelate (EVA) membrane (Leica) with frame instead of coverslip in 9-cm dishes and treated to establish three-dimensional culture as described above. The density of tumor cells seeded onto gel was adjusted to $1x10^5$. After 7-day culture, samples with EVA member were washed with PBS-DEPC and air-dried, channels formed by endothelial cells-like were selected by microscopic visualization and laser microdissected with an LCM system (Leica).

Approximately 1,500-2,000 ECs-like were laser-captured from each EVA member. The cells were immersed in digestion buffer for reverse transcription polymerase chain reaction (RT-PCR), or quantitative real-time RT-PCR.

RT-PCR and quantitative real-time RT-PCR. Total RNA was extracted from $2x10^4$ ECs-like using TRIzol reagent (Invitrogen). Aliquots of RNA were reverse transcribed to cDNA using a Superscribe First-Strand synthesis system (Invitrogen). Aliquots of cDNA were used as template for PCR to verify expression of Flk-1, vWF, CD34, AC133, Blood type B or P glycoprotein at transcription level. Thermocycling conditions were 45 sec at 95˚C, 30 sec at 58˚C, and 45 sec at 72˚C for 40 cycles, and a final extension of 72˚C for 7 min. The PCR reactions were then visualized on a 1.5% agarose gel containing 5 μg/ml of ethidium bromide after electrophoresis. The primers were as follows: Flk-1 (140 bp): 5'-ACAGTGGTATGGTTCTTGCCTCA-3', 5'-GTAGCCGC TTGTCTGGTTTGA-3'; vWF (159 bp): 5'-TCCACCGAA GCACCATCTACC-3', 5'-GAACGTAAGTGAAGCCCGA CC-3'; CD34 (150 bp): 5'-GCGCTTTGCTTGCTGAGTTT-3', 5'-TCCAAGGGTACTAGGTGTTGTAG-3'; AC133 (97 bp): 5'-CATCCACAGATGCTCCTAAGGC-3', 5'-AAGAGAAT GCCAATGGGTCCA-3'; Blood type B (189 bp): 5'-CAATG CACACTTCAACCTCTT-3', 5'-TCAGTCGGCGAATACT GTAAG-3'; P glycoprotein (174 bp): 5'-CACATGACTTT CGGCGGATGA-3', 5'-GCTGCGTTATTGGCTTCACC-3'; ß-actin (211 bp): 5'-CCTGTACGCCAACACAGTGC-3', 5'-ATACTCCTGCTTGCTG ATCC-3'.

Real-time PCR analysis was performed to quantify mRNA expression of VEGF, Flk-1, Cyclin D1, P53, and V-src on a Rotor-Gene3000 PCR system (Corbett, Australia) using the SYBR-Green PCR Master mix (Qiagen, Hilden, Germany). The PCR reaction consisted of 12.5 μl of SYBR-Green PCR Master mix, 1 μl of forward and reverse primers, and 2.0 μl of 1:10-diluted template cDNA in a total volume of 25 μl. Amplification was initiated at 50˚C for 2 min, 95˚C for 70 sec, followed by 40 rounds of 95˚C for 20 sec, 58˚C for 20 sec, and 72˚C for 30 sec. To verify that the used primer pair produced only a single product, a dissociation protocol was added after thermocycling, determining dissociation of the PCR products. The assay included a no-template control, a standard curve of four serial dilution points (in steps of 10-fold) of a cDNA mixture, and each of the tested cDNAs. All data were controlled using Rotor-Gene software (version 6.0) for quantity of RNA input by performing measurements on an endogenous reference gene (ß-actin) from the same reverse transcription reaction. Data are means from three separate experiments. The primers are as follows: VEGF (166 bp): 5'-TCACCAAGGCCAGCACATAG-3', 5'-GGGAACGC TCCAGGACTTAT-3'; Flk-1 (140 bp): 5'-ACAGTGGTA TGGTTCTTGCCTCA-3', 5'-GTAGCCGCTTGTCTGG TTTGA-3'; Cyclin D1 (171 bp): 5'-GATGCCAACCTCC TCAACGAC-3', 5'-CTCCTCGCACTTCTGTTCCTC-3'; P53 (298 bp): 5'-GCTGCTCAGATAGCGATGGTC-3', 5'-CTCCCAGGACAGGCACAAACA-3'; V-src (196 bp): 5'-CACTCGCTCAGCACAGGACAG3', 5'-AGAGGCAGT AGGCACCTTTCG-3'; ß-actin (211 bp): 5'-CCTGTACG CCAACACAGTGC-3', 5'-ATACTCCTGCTTGCTGAT CC-3'.

All human-specific primers were designed using Primer3 web software (Whitehead Institute, Cambridge, MA) and were synthesized by Sangon Biological Engineering Technology and Service Co., Ltd. (Shanghai, P.R. China).

Western blot assay. Total protein was extracted from all of transfected or non-transfected cells using Mammalian protein extraction reagent (Pierce, Rockford, IL), and an equal amount of protein from each cell line was loaded per lane and separated on a 7.5% SDS-Tris glycine PAGE gel. Gels were electroblotted onto nitrocellulose membranes (Novex, San Diego, CA) and blocked overnight by incubating with 1X Tris-buffered saline containing 0.1% Tween and 5% non-fat milk. Membranes were probed with HIF-1α (Santa Cruz) or specific-GAPDH antibody (Kangchen). The membranes were then incubated for 1 h at room temperature with horseradish peroxidase-conjugated secondary antibody. Labeled bands from washed blots were detected by ECL. Protein expression was semi-quantified using a Tiannen imager and analysis system (Shanghai, P.R. China).

Statistical analysis. ANOVA analysis or paired-samples t-test were performed to identify differences, using SPSS11.5 statistical software (Lead, US). Statistical significance was assumed at $P<0.05$, P-values presented are two-sided.

Results

Hypoxia promotes dedifferentiation in ovarian cancer cells towards ECs-like. Ovarian cancer cells were seeded and expanded on three dimensional gel exposed to 1% oxygen. In the first three days, a few tumor cells initiated to extend and reshape, gradually developed ECs-like and connected with each other, eventually forming network structures and channels by day 7 (Fig. 1A-D). Scanning electron microscopic exam demonstrated hollow tubes with diameter ranging from 8 μm to 20 μm (Fig. 1G and H). Tumor cells transfected with siRNAHIF-1α also extended, reshaped and became arc or strip-like appearances, but these cells did not form any kinds of structures or channels even over 14 days until cultures ceased (Fig. 1E and F). On the contrary, cells only grew attached to gel under normoxic conditions (21% O_2).

Characteristics of ECs-like derived from ovarian cancer cells. Antigen expression was of blood group B in SKOV-3 and P glycoprotein in ES-2 according to ATCC description. Immunofluorescence assay demonstrated that blood type B is expressed in SKOV-3 and P glycoprotein in ES-2, and EC-related markers Flk-1, AC133, CD34 and vWF are not expressed in two types of untreated ovarian cancer cells. After hypoxia promoted induction on the three-dimensional gel, ECs-like derived from SKOV-3 expressed Flk-1, AC133 and vWF except for CD34. Cells derived from ES-2 expressed Flk-1 and CD34 (Fig. 2A). ECs-like cells derived from both SKOV-3 and ES-2 expressed blood type B and P glycoprotein simultaneously under hypoxia conditions. However, tumor cells cultured under normoxic conditions or treated by HIF-1 siRNA did not express any of the markers mentioned above. We confirmed the results using RT-PCR at transcription level after ECs-like were isolated by LCM (Fig. 2B).

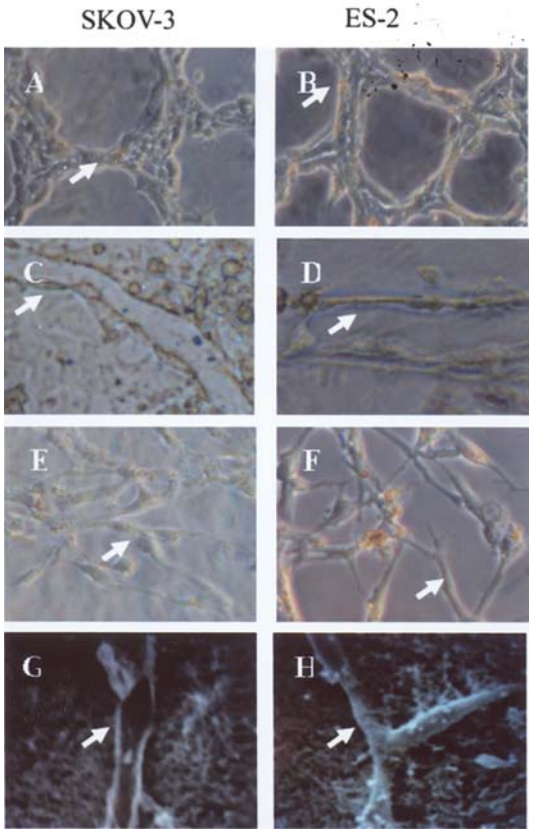

Figure 1. Hypoxia (1% O_2) induced ovarian cancer cell transendothelial differentiation and channel formation on three-dimensional gel by 7 days. A-D, tubular network and channel formation under light microscope, arrowheads point to ECs-like derived from tumor cells. E and F, tumor cells transfected with $siRNA_{HIF\text{-}1\alpha}$ showing arc or strip-like and no channel formation. G and H, tubular profiles with hollows lined by ECs-like under scanning electron microscope. Representative changes were chosen for photography (original magnification x200, A and B; x400, C-F; x1500, G and H).

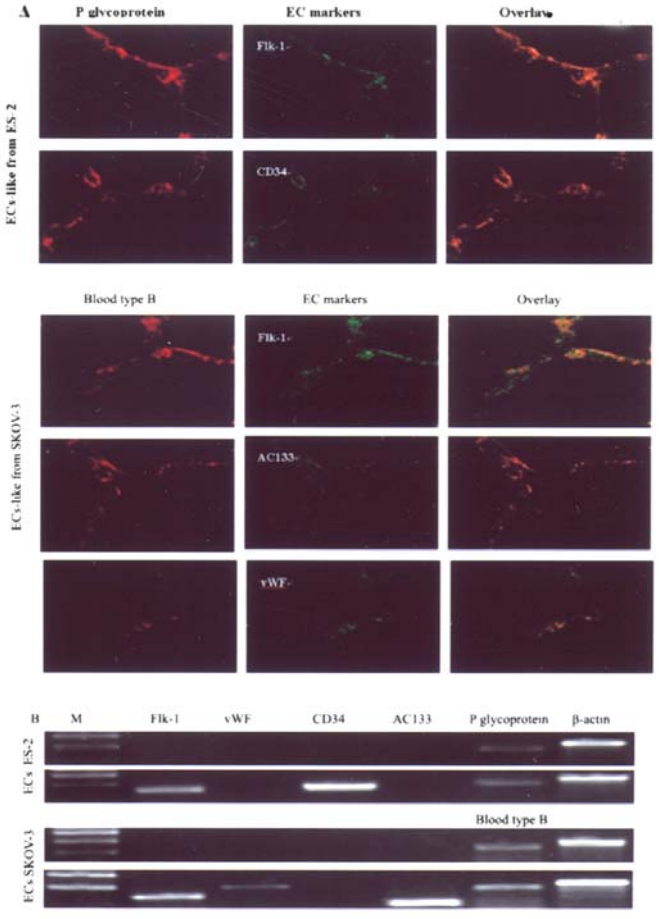

Figure 2. Immunofluorescence and RT-PCR analysis of cellular markers of ECs-like derived from ovarian cancer cells. A, lane 1 (red) shows immunofluorescence staining of P glycoprotein (ES-2) and blood type B (SKOV-3); lane 2 (green) shows immunofluorescence staining of markers associated with EC including Flk-1, CD34, AC133 and vWF. Staining intensity varied among three separate experiments. B, ECs-like were defined by RT-PCR for markers compared with initiated tumor cells.

To further define the microstructure of ECs-like, transmission electron microscopy was performed. Vacuoles and occasional granules with a single membrane which was considered as a cross section of Weibel-palade body in cytoplasm, and micro-villi outstanding of the cell surface were found while nuclei extended accordingly in ECs-like (Fig. 3A and B). These changes were not observed in tumor cells.

It is well established that EC is the main cell type responsible for acLDL uptake (27), ECs-like's capability of acLDL uptake was explored in the present study as well. Experiments showed that distribution of DiL-labeled acLDL existed in a fraction of ECs-like derived from ES-2 lining along channels (Fig. 3C and D), but not in ECs-like from SKOV-3 and other tumor cells.

Role of HIF-1α in transendothelial differentiation. Analysis of HIF-1α protein expression showed that HIF-1α is hardly expressed in SKOV-3 and ES-2 but highly expressed by 9.7- and 3.8-fold, respectively, in ECs-like induced by hypoxia on three-dimensional gel ($P<0.01$; Fig. 4A and B). Channels were inhibited consequently *in vitro* while HIF-1α were mostly down-regulated (~75% in SKOV-3 and ~68% in ES-2, $P<0.01$; Fig. 4A and B) by HIF-1α siRNA under hypoxic conditions.

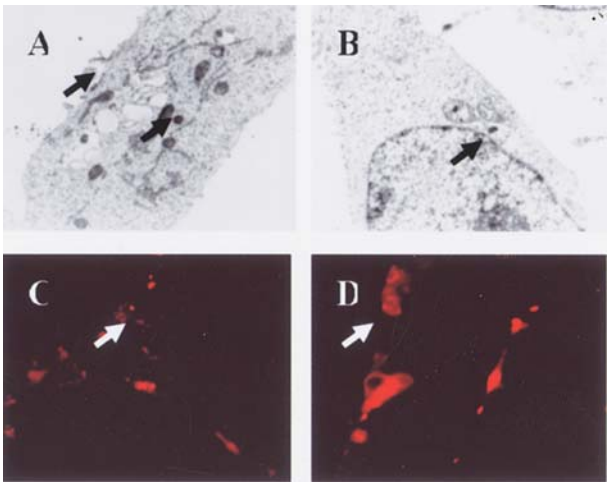

Figure 3. Ultrastructural characteristics under transmission electron microscope and capability of acLDL uptake under fluorescent microscope of ECs-like. A and B, micro-villi and Weibel-palade body indicated by arrowheads were found in ECs-like from SKOV-3 (A) and ES-2 (B) while cells extended accordingly. Poor Weibel-Palade body morphology was considered as cross section. C and D, distribution of Dil-labeled acLDL existed in fraction of ECs-like derived from ES-2 lining along channels indicated by arrowheads (original magnification x15000, A and B; x400, C and D).

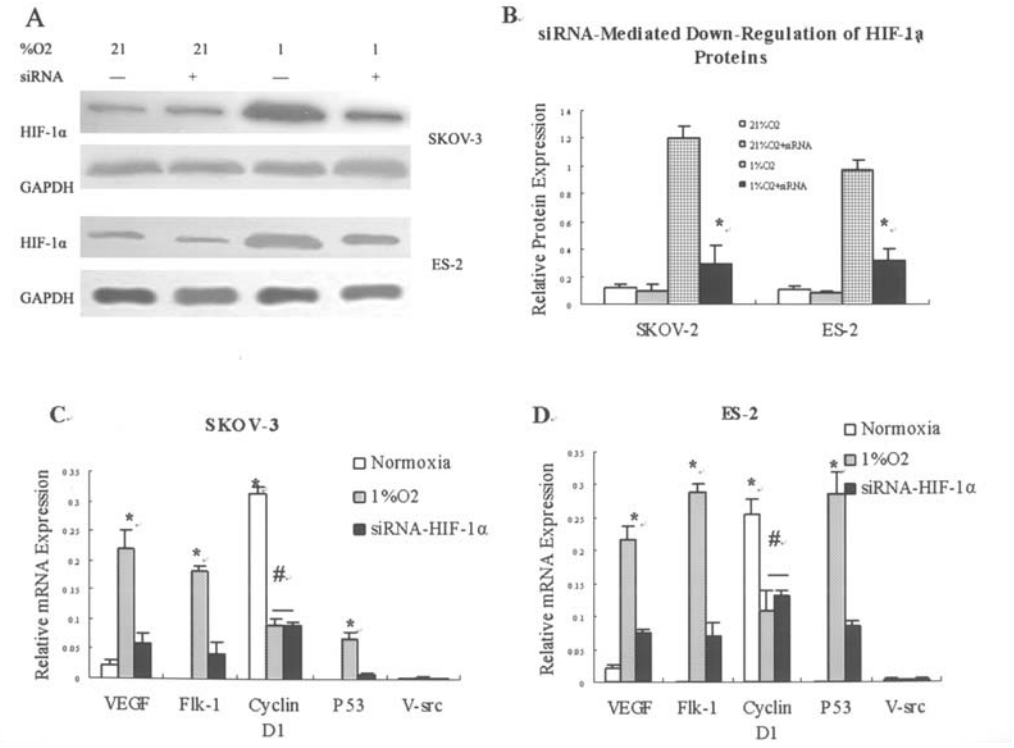

Figure 4. Effect of siRNA-HIF-1α and role of HIF-1α in transendothelial differentiation. A and B, Western blot analysis of HIF-1α proteins at the 8th day, siRNA mediated down-regulation of HIF-1α (*P<0.05). C and D, analysis of real-time RT-PCR at the 8th day revealed that hypoxia increased mRNA expression of VEGF, Flk-1 and P53, and decreased expression of Cyclin D1 during transendothelial differentiation, these changes were inhibited effectively by RNA interference of HIF-1α, but no significant difference of Cyclin D1 was shown with siRNA-mediated inactivation of HIF-1α. No cells expressed V-src mRNA under all conditions. Data shown are the mean results ± SD of three separate experiments (*P<0.01; #P>0.05).

In an attempt to gain specific insight into the mechanisms of hypoxia induction, we further examined hypoxia response gene mRNA levels including VEGF, Flk-1, Cyclin D1, P53 and V-src of ECs-like isolated by LCM while siRNA was used to inactivate HIF-1α. Hypoxia strongly promoted VEGF and Flk-1 mRNA expression in ECs-like when compared to two cancer cells *in vitro* (9.2-10.2- and 18.3-28.8-fold, respectively, all P<0.01; Fig. 4C and D). The effect was inhibited effectively by HIF-1α siRNA under hypoxia. The inhibition of VEGF was ~72% in SKOV-3 and ~65% in ES-2, and of Flk-1 was ~77% in SKOV-3 and ~76% in ES-2 (P<0.01; Fig. 4C and D). Analysis revealed that mRNA expression of Cyclin D1 was high in SKOV-3 and ES-2, and decreased to 29.4-42.2% of ECs-like (P<0.01; Fig. 4C and D), this change was not recovered by inhibition of HIF-1α expression induced by hypoxia (Fig. 4C and D). P53 showed significantly high expression in both ECs-like while it was not expressed in SKOV-3 and ES-2 (P<0.01; Fig. 4C and D). Inhibition of HIF-1α expression was sufficient to block hypoxia-induced P53 mRNA expression (~88% in SKOV-3 and ~70% in ES-2, P<0.01; Fig. 4C and D). Our data further showed that no cells expressed V-src mRNA under normoxic or hypoxic conditions.

Discussion

The evidence shown previously demonstrated the powerful influence of the microenvironment on the transendothelial differentiation of aggressive melanoma cells (24). It is known that polymer scaffolds under compressive stress can promote cell differentiation (25). Hypoxia is a major physiologic stimulus for angiogenesis in oncology (9). The present study challenged ovarian cancer cells to differentiate into ECs-like and form channels using an environment with low oxygen tension on scaffolds. Ovarian cancer cells were successfully engaged in network formation surrounding spheroids of tumor cells in three-dimensional culture (22), these quiescent cellular spheroids are analogous to those regions within tumors, resulting in hypoxia (28,29). This strongly implies that the effect of hypoxia in the process can not be excluded. A recent study also found that expression of genes such as laminin5γ2, EphA2, and Tie-1, which were involved in vasculogenic mimicry, were significantly increased in tumor cells under hypoxia (20). The present study demonstrates that hypoxia has a strong induction influence in the event of ECs-like differentiation of ovarian cancer cells.

HIF-1 has been identified as a master regulator of the transcriptional response to oxygen deprivation (9). HIF-1 is composed of two subunits, HIF-1α and HIF-1ß. HIF-1α is the key regulatory component which controls the expression of a variety of genes related tumor biology (17). Channel mimicry by ECs-like was not promoted by interference with the expression of HIF-1α by siRNA. This demonstrates that HIF-1 modulation is crucial in ovarian cancer cell trans-differentiation towards ECs-like induced by hypoxia. At the molecular level, HIF-1 activates the response of a large assortment of downstream genes to cellular hypoxia. Hypoxic induction of VEGF appears central to tumor angiogenesis (30), VEGF is a potent and specific mitogen for EC and its high affinity receptor Flk-1 is considered to be the dominant

signaling receptor for EC differentiation in angiogenesis (31,32). The study showed that hypoxia strongly promoted VEGF and Flk-1 mRNA expression in ECs-like different from SKOV-3 and ES-2. Previous study reported that Flt-1 but not Flk-1 is up-regulated by hypoxia at the transcriptional level (31). The present results show that expression of VEGF and Flk-1 can be inhibited while HIF-1α is silenced. Since VEGF can up-regulate Flk-1 (33,34), the up-regulation of Flk-1 may be related to VEGF expression dependent of HIF-1α. The tumor suppressor P53 has been shown to accumulate under conditions of severe hypoxia, and evidence now links oncogene V-src with angiogenesis stimulated by hypoxia (35,36). This demonstrates that transcriptional activation of P53 via HIF-1 participates in the differentiation of ovarian cancer cells into ECs-like while V-src is silenced.

Cyclin D1 is associated with inactivation of the tumor suppressor pRB, shortened duration of the G_1 phase and tumor malignant transformation, but the exact manner is still obscure (37,38). This study showed that Cyclin D1 expression of ECs-like decreased significantly after oxygen deprivation. The changes were not recovered by inhibition of HIF-1α expression. Consistent with the present study, a study showed that high concentrations of HIF-1α were not associated with the G_1-phase protein Cyclin D1 in invasive breast cancer (38). The study demonstrates that the decrease of Cyclin D1 contributes to ovarian cancer cell transendothelial differentiation independent of HIF-1, and the exact mechanism requires further investigation.

ECs display some unique characteristics, including the expression of surface markers such as Flk-1, CD34, vWF, platelet endothelial cell adhesion molecule (PECAM), Tie-1, and Tie-2, the presence of Weibel-Palade bodies, and the capacity to incorporate acLDL (39,40). The results showed that ECs-like derived from SKOV-3 or ES-2 can express Flk-1 or CD34, or vWF, form Weibel-Palade body-like organelles, and uptake acLDL. This study demonstrates that ECs-like derived from epithelial ovarian cancer cells present some similar endothelial-specific characteristics. Furthermore, Flk-1 appears to be the dominant signaling receptor in VEGF-induced mitogenesis, which is specifically expressed on ECs (41). The finding that ECs-like from both SKOV-3 and ES-2 express Flk-1 strongly implies the importance of Flk-1 in transendothelial differentiation as well.

The notion that tumors might contain 'cancer stem cells', rare cells with indefinite proliferative potential, that drive the differentiation and self-renewal provides new insights into cancer biology (42). The overall data suggest that CD34 and AC133 could be specific markers for various stem and progenitor cell populations, and $CD34^+$ or/and $AC133^+$ cells from bone marrow as a subpopulation of cells could differentiate into ECs (43-45). The study showed that ECs-like can express AC133 or CD34 after hypoxic deprivation, which suggested that these ECs-like might derive from ovarian cancer cells with stem cell characteristics, but further investigations are warranted to confirm this. Identification of the differences between endothelial precursor cells (EPCs) and mature ECs indicated that both cells may express some common endothelial-specific markers, including Flk-1, and vWF, and they also have the capacity to incorporate ac-LDL (40). Partial marker expression of EPC can be lost or appear once the EPCs differentiate into more mature ECs, and AC133 is rarely found on mature ECs (39,40). The present results demonstrate that ECs-like derived from epithelial ovarian cancer cells share similar characteristics with EPCs rather than ECs. This encourages further investigation into the potential relevance of a 'plastic' tumor-cell phenotype, and challenges our current knowledge of how to identify and target tumor cells that can masquerade as other cell types.

Acknowledgments

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