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Research Article

ES-2 Ovarian Cancer Cells Present a Genomic Profile Inconsistent with their Reported History

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Citation: Eric J Devor, Jace R Lapierre, David P Bender. ES-2 Ovarian Cancer Cells Present a Genomic Profile Inconsistent with their Reported History. Obstetrics and Gynecology Research 4 (2021): 233-238.

Abstract

ES-2 ovarian cancer cells have long been reported to have originated from a primary clear cell carcinoma of the ovary presenting in a 47 year-old African American patient. Two recent publications have offered evidence calling both of these characteristics into question. Our objective was to further study this cell line using quantitative real-time PCR (qPCR) and mitochondrial DNA (mtDNA) sequencing in order to confirm or refute these inconsistencies. qPCR assays on two characteristic loci, hepatocyte nuclear factor 1β (NHF-1β) and glutathione peroxidase 3 (GPX3), suggest that ES-2 are unusual clear cell carcinoma cells that appear more like high grade serous carcinoma than clear cell. Further, mtDNA haplotyping places the ancestral origin of the patient's lineage in the Middle East or Europe and not Africa. These results are consistent with and support the conclusions of the two recent publications.

1. Introduction

The cell line ES-2 (RRID:CVCL_3509) is reported to be derived from a poorly differentiated clear cell carcinoma of the ovary (OVCCC) presenting in a 47-year-old African American patient [1]. In 2014, Kwok and colleagues [2] demonstrated that ES-2 cells displayed immunohistochemical staining for hepatocyte nuclear factor 1β (HNF-1β) and Wilm's tumor 1 (WT1) in ES-2 cells that was more like that

seen in OVCAR3 cells, a high grade serous ovarian carcinoma, than in three well known OVCCC cell lines; OVMANA, OVISE and OVSAYO. In addition, evidence indicating that the ancestry of the ES-2 cell line is 58.8% Southern European, 27.1% Northern European and only 2.6% African has been presented [3]. These data call into question what ES-2 cells really are. Here we present additional data based upon qPCR assays and mitochondrial DNA (mtDNA) haplotyping that suggest ES-2 cells are clearly not typical OVCCC and likely not of purely African ancestry.

2. Methods

2.1 Cell line propagation and validation

ES-2 cells were propagated in McCoy's 5A media with 10% FBS and 1% pen/strep antibiotic at $37^{\circ}C$ and 5% CO_2. Validation of cells consisted of purifying genomic DNA (gDNA) using the DNeasy Blood and Tissue Kit (QIAGEN) following manufacturer's recommendations and sending aliquots of that gDNA to BioSynthesis (Lewisville, Texas) for CODIS genotyping. Total cellular RNA was purified using the mirVana RNA kit (Thermo Fisher) following manufacturer's recommendations. Yield and purity of the RNAs was assessed on an Agilent Model 2100 Bioanalyzer and a Trinean DropSense 16. These assessments were carried out in the Genomics Core Facility of the Iowa Institute of Human Genetics (IIHG). RNAs were regarded as acceptable if the RNA integrity number (RIN) [4] was at least 9.0 with minimal non-RNA components present.

Additional human cell lines used in this study are the ovarian cancer cells OVCAR-3 (RRID:CVCL_0465), MDAH-2774 (RRID:CVCL_0420), TOV-112D (RRID:CVCL_3612) and the cervical cancer cell line

HeLa (RRID:CVCL_0030). All cells were propagated in optimum media according to source recommendations; ATCC for OVCAR-3, TOV-112D and HeLa and AddexBio for MDAH-2774. All cell lines have recently been validated (October, 2021) via STR profiling at BioSynthesis (Lewisville, Texas). Total RNA and gDNA purifications from these cells were carried out as described above. Finally, all cell lines are mycoplasma-free.

2.2 Quantitative real-time PCR

Gene-specific qPCR was carried out by first reverse transcribing purified and QC validated total RNA from ES-2 cells using the SuperScript III RT kit (Thermo Fisher). Resulting cDNAs were amplified in the presence of SYBR Green using the primer sequences presented in Table 1. All amplifications were performed on an Applied Biosystems 5700HT platform in the Genomics Core Facility of the Iowa Institute of Human Genetics (IIHG). Gene-specific expression was then normalized against 18S rRNA (Table 1) to generate ΔCt values. Comparisons of fold changes among ovarian cancer cell lines were carried out using the standard $\Delta\Delta Ct$ method [5, 6]. Significance of fold changes was evaluated using a standard t-test with unequal variances [7]. A value of $p < 0.05$ was regarded as significant.

2.3 Mitochondrial DNA (mtDNA) haplotyping

A 428bp segment of hypervariable region 1 (HVR1) of the human mitochondrial genome was amplified using the primer sequences shown in Table 1. Amplicons were purified using the QIAquick PCR Purification Kit (QIAGEN) according to manufacturer's recommendations. Purified amplicons were sequenced on an Applied Biosystems Model 3730xl platform in the Genomics Core Facility of the Iowa Institute of Human Genetics (IIHG). The mtDNA

haplotype was determined using a MITOMASTER   search in MITOMAP (mitomap.org).

Locus	Sequence	Amplicon (bp)	Tm (^{o}C)
CDKN1A (p21)	For: TGAGTTGGGAGGAGGCA	226bp	55.8
	Rev: GCTTCCTCTTGGAGAAGATCAG		54.9
CCNE1 (cyclin E1)	For: CCAGGAAGAGGAAGGCAAAC	228bp	55.2
	Rev: GCCTCTGGATGGTGCAATAA		55.1
HNF-1β	For: GCCCACACACCACTTACTTCG	132bp	58.3
	Rev: GTCCGTCAGGTAAGCAGGGAC		59.6
GPX3	For: TACGGAGCCCTCACCATTGATG	155bp	58.9
	Rev: CAGACCGAATGGTGCAAGCTCT		59.7
18S rRNA	For: AACCTTTCGATGGTAGTCGCCG	104bp	59.4
	Rev: CCTTGGATGTGGTAGCCGTTT		57.6
mtDNA HVR1	For: TCCACCATTAGCACCCAAAGCTA	428bp	58.4
	Rev: ATTGATTTCACGGAGGATGG		52.6

Table 1: PCR and qPCR Primer Sequences Used in This Study.

3. Results

3.1 CODIS validation

We routinely CODIS verify all of the cancer cell lines in our laboratory. A fifteen locus CODIS genotype profile of our ES-2 cells was compared with profiles provided by ATCC, Cellosuarus and ADDEXBIO, two of which are commercial sources of these cells. No discrepancies were seen. This supports a conclusion that our cells are, in fact, ES-2 cells as reported in several sites.

3.2 Quantitative PCR

Ovarian clear cell carcinomas and cell lines present a genomic and expression profile distinct from other ovarian cancer histologies. Unlike other histologies, OVCCC have a very low TP53 mutation prevalence and elevated expression of both p21 (CDKN1A) and cyclin E1 (CCNE1) [8]. Further, OVCCC has characteristically high expression of glutathione peroxidase

3 (GPX3) and hepatocyte nuclear factor -1β (HNF-1β) [8]. We confirmed that the cells we were using contained a TP53 mutation, S241F, as reported elsewhere. Using OVCAR3 high grade serous ovarian cancer cells and MDAH-2774 and TOV-112D ovarian endometrioid adenocarcinoma cells as references we found that ES-2 cells expressed both CDKN1A (p21) and CCNE1 (cyclin E1) at similar levels (Table 2). HNF-1β is expressed at much higher levels than in MDAH-2774 and TOV-112D cells but, consistent with a previous report [2], ES-2 HNF-1β was slightly lower than that in OVCAR3 cells (Table 2). Similarly, GPX3 expression was much higher than in MDAH-2774 and TOV-112D cells but enormously lower than in OVCAR3 cells (Table 2). This expression profile, particularly for the presumeably diagnostic HNF-1β and GPX3 loci, suggests that ES-2 are not typical OVCCC cells.

3.3 mtDNA haplotyping

The mitochondrial HVR1 sequence from ES-2 is identified in MITOMASTER as belonging to the N1b clade. This clade is historically found in the Middle East and Europe. This is consistent with the ancestry reported for this cell line by Dutil and colleagues [3]. A similar analysis of OCVAR3 cells placed them in the European clade H2a, the MDAH-2774 cells in the European U4a clade and the TOV-112D cells in the European K1a clade. These placements are all consistent with their reported ancestries. As a control, we also sequenced an HVR1 mtDNA amplicon from HeLa cells. This sequence was assigned to the African clade L3b, consistent with their ancestry and the ethnicity of the well-known donor of those cells.

	OVCAR3	MDAH-2774	TOV-112D
CDKN1A:			
ES-2	-1.39	3.96	2.7
CCNE1:			
ES-2	-5.68	4	2.64
HNF-1β:			
ES-2	-2.31	65.55	6.1
GPX3:			
ES-2	-1226.43	6.7	5.4

Table 2: Quantitative PCR Comparison of ES-2 with OVCAR3, MDAH-2774 and TOV-112D ovarian cancer cell lines. Values represent fold changes with positive values being higher expression in ES-2 cells and negative values being lower expression in ES-2 for each cell line pair. All comparisons were statistically significant at $p < 0.05$.

4. Conclusions

It must be emphasized that the data presented here and the questions raised do not in any way diminish the utility of ES-2 ovarian cancer cells as a resource for *in vitro* studies. However, they do support a previous caution regarding their use as a model for OVCCC histology [2]. Clearly, studies involving cultured cells should examine those cells beyond simply assessing their CODIS genotypes and reported histologic source. Doubtless, however, the vast majority of cultured cancer cells are what they are supposed to be but it's best to be sure. Finally, the ancestry estimating that we quote here came from a much larger study involving nearly 1400 cancer cell lines including 65 ovarian and 29 endometrial lines. One striking fact that stood out was the dearth of cancer cell lines from patients with African ancestry.

Indeed, only 82 cell lines in that report (5.9%) displayed an African ancestry greater than 50%. Of the ovarian cancer cells only 3 (4.6%) displayed an African ancestry greater than 50% and none of the endometrial cancer cells. This is a short-fall that should be addressed. It has been pointed out that there is a significantly greater prevalence of more aggressive subtypes of endometrial cancers among non-Hispanic Black (NHB) women as compared with non-Hispanic White (NHW), Hispanic and Asian

women [9]. This fact translates into significantly lower survival among NHB women diagnosed with endometrial cancer [9-11]. While factors including socio-economic status and access to healthcare are undoubtedly contributing to differences in prevalence and survival, they cannot account for the differences in histology.

The situation in ovarian cancer is much the same in that NHB women have an overall lower survival rate compared with NHW women [12]. Clearly there are racial/ethnic differences in gynecologic cancer incidence, survival and histologic profiles. Since it is likely that some of the reasons for this will be found in examining various gynecologic cancer cell lines, Dutil and colleagues [3] have performed a valuable service by providing the ancestral composition of many of the cell lines that will be used. It has also served to point out that cell lines established from patients with African ancestry are woefully under-represented or not represented at all.

Acknowlegements

Conflict of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data used in this study are available from the authors upon reasonable request.

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