Gynecologic Oncology 138 (2015) 620–626

Contents lists available at ScienceDirect

Gynecologic Oncology

journal homepage: www.elsevier.com/locate/ygyno

Identification of potential therapeutic targets by molecular profiling of 628 cases of uterine serous carcinoma

Nathaniel L. Jones [a], Joanne Xiu [b], Sandeep K. Reddy [b], William M. Burke [a], Ana I. Tergas [a], Jason D. Wright [a], June Y. Hou [a,*]

[a] Columbia University College of Physicians and Surgeons, New York Presbyterian Hospital, United States
[b] Caris Life Sciences, United States

HIGHLIGHTS

- Our molecular analysis on the largest cohort of USC to date confirmed that USC is a genetically heterogeneous disease.
- Rational, pathway driven treatments may offer alternative strategies to treat patients with uterine serous carcinoma.

ARTICLE INFO

Article history:
Received 20 April 2015
Received in revised form 17 June 2015
Accepted 22 June 2015
Available online 26 June 2015

Keywords:
Uterine
Serous
Endometrial
Molecular
Genomic
Carcinoma

ABSTRACT

Background. Therapeutic options are limited for uterine serous carcinoma (USC). *TP53*, *PIK3CA*, *FBXW7*, and *ERBB* mutations, as well as HER2 and EGFR overexpression have been reported. We aim to evaluate patterns of molecular, genomic and protein changes in 628USC tumors.

Methods. 628 consecutive cases of USC submitted to Caris Life Sciences from Mar, 2011 to July, 2014 were reviewed. These were analyzed using the Illumina TruSeq Amplcon Cancer panel to search for sequenced variants in 47 genes commonly implicated in carcinomatosis. In situ hybridization and immunohistochemistry were also used to assess copy number and protein expression, respectively, of selected genes.

Results. 31 out of 47 genes of interest harbored mutations, including TP53 (76%), PIK3CA (29%), FBXW7 (12%) and KRAS (9.3%). BRCA1 and BRCA2 were mutated in 9.1% and 6.3%, respectively. ERCC1 and MGMT were absent in 81% and 46% of tumors analyzed, respectively, suggesting potential benefit from platinum and alkylating agents. While not traditionally considered hormone-dependent, our cohort showed high ERα (60%), PR (32%), and AR (27%) expression. HER2 overexpression was 10% via IHC, amplification was 17% via CISH/FISH and mutation was 2% via NGS. While low in PTEN mutation frequency (7%), 45% of USC showed PTEN loss on IHC, and 29% harbored PIK3A mutation, suggesting deregulation of P13K/AKT pathway in a subset of patients. 11% expressed PDL1 and 67% expressed PD1.

Conclusions. Our findings suggest hormonal receptors, as well as genes implicated in DNA repair, cell proliferation and cell cycle pathways are of interest in USC.

Published by Elsevier Inc.

1. Introduction

In the United States endometrial cancer (EC) is the most common gynecologic malignancy and represents the fourth most common cause of cancer among women. It is estimated that 52,000 new cases will be diagnosed in 2014 and that 8500 women will die of their disease [1]. Endometrial carcinoma is traditionally divided into Type I (endometrioid) and Type II (non-endometrioid) disease based on clinical and pathologic attributes. Type I EC accounts for 80–90% of new cases and most often occurs in younger women. These tumors are hormonally dependent, and the vast majority is associated with a favorable prognosis. Type II disease, which consists of highly aggressive variants such as uterine serous cancer (USC), accounts for 39% of all EC-related deaths largely due to biologic aggression and chemo-resistance [2]. Due to its distinct biologic behavior, USC is typically excluded from large clinical trials. Currently, the standard of care for patients with USC is cytoreductive surgery followed by platinum-based chemotherapy and/or radiotherapy: a recommendation that remains largely extrapolated from data on high-grade serous ovarian cancer and advance stage type I EC. Despite aggressive therapy, prognosis is poor. Five year disease-specific survival for advanced stage disease is 33% [3].

Recently, The Cancer Genome Atlas (TCGA) Research Network proposed a reclassification of EC into four subgroups based on in-depth

* Corresponding author at: Division of Gynecologic Oncology, Herbert Irving Pavilion, 161 Fort Washington Ave, Suite # 837, New York, NY 10032, United States.
E-mail address: jh3558@cumc.columbia.edu (J.Y. Hou).

http://dx.doi.org/10.1016/j.ygyno.2015.06.034
0090-8258/Published by Elsevier Inc.

genome-wide analysis of more than 350 patients. Their findings suggest few copy number variations, significant microsatellite instability (MSI), mutations in POLE, a subunit of DNA polymerase Epsilon, and increased activation of WNT/CTNNB1 pathway in endometrioid EC. TCGA and other groups also reported frequent mutations in the phosphatidylinositol-3-OH kinase (PI3K) pathway, including PTEN, PIK3CA, and PIK3R1 genes in the neoplastic process of type I EC, as well as mutations in FGFR2, CTNNB1, ARID1A, and KRAS [4–7]. Whole exome sequencing studies have shown significant mutations in chromatin remodeling and ubiquitin ligase complex genes [8] as well as DNA damage, cell cycle, and cell proliferation genes [9].

USC harbors a distinct genomic signature from other EC histologic subtypes, with reported overlaps between USC and high-grade serous ovarian carcinoma and basal-like breast cancer [7]. There is a tremendous effort to optimize outcome of patients with USC by tailoring treatments based on genomic alterations. Collaborative efforts through TCGA, as well as other investigators reports frequent mutations in TP53, PIK3CA, ERBB2, FBXW7, PPP2R1A and ARID1A. These genes implicated in cell cycle regulation, cellular proliferation and chromatin remodeling pathways can be potentially therapeutically targetable [6–15]. There is increasing knowledge of the molecular changes that contributes to USC carcinogenesis. However, our understanding is limited due to sample size of most of the studies. To that end, we have reviewed and analyzed multiplatform profiles of over 600 USC tumors with an emphasis on identifying pathway signatures and associated molecular biomarkers for potential therapeutic interventions.

2. Materials and methods

2.1. Tissue samples

Approval was granted from the Institutional Review Board at Columbia University Medical Center. 3133 consecutive formalin-fixed, paraffin-embedded tissue (FFPE) cases (March 2011 to July 2014) of EC were submitted to a CLIA-certified molecular profiling laboratory (Caris Life Sciences, Phoenix, AZ). H&E slides were prepared for each tumor sample and were reviewed by board-certified pathologists to verify the diagnosis on the pathology reports accompanying the tumor samples. 628 cases of USC were confirmed. Analysis was performed on multiplatform profiling for these USC cases, which included a combination of immunohistochemistry (IHC), in situ hybridization (ISH), and sequencing. Researchers were blinded from patient identifiers and analyses were performed on de-identified data.

2.2. Immunohistochemistry

IHC was performed on FFPE tumor samples using commercially available detection kits, automated staining techniques (AutostainerLink 48, Dako, Carpinteria, CA; and Benchmark XT, Ventana, Tucson, AZ), and primary antibodies for androgen receptor (AR27), estrogen receptor (ER-SP1), human epidermal growth factor receptor 2 (Her2-4B5), DNA excision repair protein (ERCC1-8F1), O(6)-methylguanine-methyltransferase (MGMT-MT23.3), P-glycoprotein (PGP-C494), progesterone receptor (PR-1E2/100), phosphatase and tensin homolog (PTEN-6H2.1), ribonucleotide reductase M1 (RRM1-polyclonal), serum protein acidic and rich in cysteine (SPARC monoclonal-12251), serum protein acidic and rich in cysteine (SPARC polyclonal), topoisomerases 1 (TOPO1-1D6), topoisomerases 1 and 2α (TOPO2α-3 F6), thymidylate synthase (TS106/4H4B1), MET proto-oncogene, receptor tyrosine kinase (cMET-SP44), tubulin beta-3 chain (TUBB3-polyclonal), transducin-like enhancer of split 3 (TLE3-polyclonal), programmed cell death protein 1 (PD1-MRQ-22), and programmed death-ligand 1 (PD-L1-130021). Scoring system and thresholds for all antibodies are provided (Supplementary Table 1).

2.3. In situ hybridization

Fluorescent ISH (FISH) and chromogenic ISH (CISH) were utilized. FISH was performed to detect HER-2/neu [HER-2/CEP17 probe], TOP2A [TOP2/CEP17 probe] and cMET [cMET/CEP7 probe] gene amplification (all Abbott Molecular/Vysis). CISH was also used for Her-2/neu (INFORM HER-2 Dual ISH DNA Probe Cocktail) and cMET (Ventana) (See Supplemental Fig. 1 for example). HER2 amplification: 20 nuclei of the target area were scored and the ratio of h*er2* to chromosome 17 was determined; if Her2/chr17 ratio ≥2.0, Her2 was amplified, if the ratio was <2.0, Her2 was not amplified. If the result was 1.8 to 2.2, another target area containing an addition 20 nuclei was scored and the results were combined from both test areas.

TOP2A amplification: defined as TOP2A/CEP17 ratio ≥2.0. HER-2/CEP17 ratio ≥2 was considered amplified.
cMET amplification: defined as ≥ 5 copies per tumor cells were detected on average.

2.4. Mutational analysis

2.4.1. DNA extraction

DNA for mutation analysis was extracted from FFPE tissues after macrodissection of the fixed slides in an area that % tumor nuclei ≥ 10% as determined by a pathologist. DNA was extracted using the QIAamp DNA FFPE Tissue kit according to the manufacturer's instructions (QIAGEN Inc., Valencia, CA).

2.4.2. Next-generation sequencing (NGS)

NGS was performed on genomic DNA using the Illumina MiSeq platform. No DNA from matched normal tissue was sequenced. Specific regions of 47 genes of interest related to cancer genomics based on current literature were amplified using a modified Illumina TruSeq Amplicon Cancer panel (Supplementary Tables 2–3). All variants reported are detected with >99% confidence based on the mutation frequency present and the amplicon coverage. A two-sided binomial calling model was used and calling threshold for mutation was 10% variant frequency, allowing for calling mutation and wild type (for regions covered) at 99% confidence. Variants known to be pathogenic, presumed to be pathogenic, and those of unknown significance were included in the analysis.

2.4.3. Sanger sequencing

Sanger sequencing was performed in a small portion of tumors before NGS was routinely available. Sanger sequencing included selected regions of BRAF, KRAS, c-KIT, EGFR, NRAS, and PIK3CA and was performed by using M13-linked PCR primers designed to flank and amplify targeted sequences. Sequence traces were analyzed using Mutation Surveyor software v3.25 (Soft Genetics). All sequence traces were reviewed by two technologists and results were confirmed by a board certified clinical molecular geneticist (Additional information and examples of trace sequences included in Supplemental Table 4 and Supplemental Fig. 2). A sample was deemed "mutated" if the same nucleotide change is identified in both the forward and reverse traces as well as being absent in the control sample traces. All mutation nomenclature adhered to the guidelines set forth by the Human Genome Variation Society.

2.5. Statistical methods

Differences in proportions of positive results were calculated by two-tailed Fisher Exact tests. Tests with indeterminate results were not counted towards to the total sample number.

3. Results

Six hundred twenty eight cases of USC were identified among the 3133 cases of EC submitted. The median age was 67, with the majority