ONCOLOGY

Differential sensitivity to platinum-based chemotherapy in primary uterine serous papillary carcinoma cell lines with high vs low HER-2/neu expression in vitro

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OBJECTIVE: We sought to identify effective chemotherapy regimens against uterine serous papillary adenocarcinoma (USPC).

STUDY DESIGN: Six USPC, half of which overexpress HER-2/neu at 3+ level, were evaluated for growth rate and in vitro sensitivity to 14 single-agent chemotherapies and 5 combinations by ChemoFx (Precision Therapeutics Inc, Pittsburgh, PA).

RESULTS: Cell lines overexpressing HER-2/neu showed higher proliferation when compared to low HER-2/neu-expressing cell lines and a lower half maximum inhibitory concentration (IC_{50}) when exposed to the majority of single-agent chemotherapies. High HER-2/neu expressors were more sensitive to platinum compounds, manifesting a 5.22-fold decrease in carboplatin-IC_{50} ($P = .005$) and a 5.37-fold decrease in cisplatin-IC_{50} ($P = .02$). When all cell lines were analyzed as a group, chemotherapy agents tested demonstrated lower IC_{50} when used in combination than as individual agents.

CONCLUSION: USPC overexpressing HER-2/neu display greater in vitro sensitivity to platinum compounds when compared to low HER-2/neu expressors. Higher proliferative capability rather than increased drug resistance may be responsible for the adverse prognosis associated with HER-2/neu overexpression in USPC.

Key words: chemoresistance, chemosensitivity, endometrial neoplasms, HER-2/neu, uterine serous tumors

Cite this article as: Cross SN, Cocco E, Bellone S, et al. Differential sensitivity to platinum-based chemotherapy in primary uterine serous papillary carcinoma cell lines with high vs low HER-2/neu expression in vitro. Am J Obstet Gynecol 2010;203:162.e1-8.

Endometrial cancer is the most common female genital tract malignancy in the United States, with an incidence of 42,160 new cases and 7780 deaths annually.[1] Two types of endometrial carcinoma, namely type I and II tumors, have been described, based on both clinical and histopathological variables.[2] Type I endometrial cancers, which account for the majority (about 80%) of cases, are usually well or moderately differentiated and endometrioid in histology. These neoplasms are frequently diagnosed in younger women, are associated with a history of hyperestrogenism as the main risk factor, and typically have a favorable prognosis with appropriate therapy. In contrast, type II endometrial cancers are poorly differentiated tumors, or tumors with serous papillary or clear cell histology. Although type II tumors account for only a minority of endometrial cancers, most recurrences and deaths occur in this group of patients.

Uterine serous papillary adenocarcinoma (USPC), which accounts for about 10% of endometrial cancers, has a propensity for early intraabdominal and lymphatic spread even at presentation and is characterized by a highly aggressive biologic behavior.[3] Unlike the histologically similar high-grade ovarian cancer, USPC is a chemoresistant disease from onset, with in vivo responses to combined cisplatin-based chemotherapy in the order of 20% and of short duration.[4-6] Our group has recently reported HER-2/neu overexpression by immunohistochemistry (IHC) and amplification of the c-erbB2 gene by fluorescent in situ hybridization (FISH) in a large percentage of patients harboring USPC.[7-9] These findings, recently confirmed by other groups[10] including the Gynecologic Oncology Group in a cooperative multicentric study,[11] have identified HER-2/neu overexpression in USPC as an independent variable associated with poor outcome, and one that occurs more frequently in African American women than in Caucasian women.[7-9]

Although HER-2/neu overexpression has been previously associated with resistance to chemotherapy and poor survival in multiple human malignancies

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Received Nov. 25, 2009; revised Jan. 13, 2010; accepted Feb. 22, 2010.

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Supported in part by grants from the Angelo Nocivelli, the Berlucchi, and the Camillo Golgi Foundation, Brescia, Italy; National Institutes of Health (NIH) R01 CA122728-01A2 (Dr Santin); and Grants 501/A3/3 and 0027557 from the Italian Institute of Health (Dr Santin). This investigation was also supported by NIH research Grant no. CA-16359 from the National Cancer Institute.

TABLE 1
Patient characteristics

Patient	Age, y	Race	FIGO stage	USPC histopathology	Year of diagnosis
USPC ARK-1	62	AA	IVA	Pure	1997
USPC ARK-2	63	AA	IVB	Pure	1998
USPC ARK-3	59	AA	IVB	Mixed	2006
USPC ARK-4	73	C	IVB	Pure	2004
USPC ARK-5	73	AA	IIIC	Pure	2006
USPC ARK-6	62	C	IB	Mixed	2005

AA, African American; *C*, Caucasian; *FIGO*, International Federation of Gynecology and Obstetrics; *USPC*, uterine serous papillary adenocarcinoma.

Cross. Effective chemotherapy regimens against USPC. Am J Obstet Gynecol 2010.

including breast,[12] ovarian,[13] and endometrial carcinoma,[14] to our knowledge, no study has carefully evaluated the in vitro chemosensitivity/chemoresistance of this highly aggressive variant of endometrial cancer. To fill this gap in knowledge, we used ChemoFx (Precision Therapeutics Inc, Pittsburgh, PA) to analyze the in vitro sensitivity of 6 primary USPC cell lines recently established and characterized in our laboratory[15] to 14 standard single-agent chemotherapies and 5 combination chemotherapies.[16] Half the cell lines selected overexpress HER-2/neu at 3+ levels and harbor amplification of the c-erbB2 oncogene by FISH. Surprisingly, although in vivo HER-2/neu overexpression is correlated with a more aggressive disease in patients with USPC,[8,9] growth-inhibition data suggest that these tumors display greater in vitro chemosensitivity to platinum compounds and higher proliferative capability when compared to HER-2/neu-negative tumors.

TABLE 2
HER-2/neu expression in primary uterine serous papillary adenocarcinoma cell lines

Sample	IHC	FISH	RT-PCR mRNA copy no.
Control			1
USPC ARK-1	3+	2.5	373
USPC ARK-2	3+	5.2	607
USPC ARK-3	3+	4.7	677
USPC ARK-4	0	1.6	7
USPC ARK-5	0	1.4	13
USPC ARK-6	1+	0.9	6

FISH, fluorescent in situ hybridization; *IHC*, immunohistochemistry; *mRNA*, messenger RNA; *RT-PCR*, real-time polymerase chain reaction; *USPC*, uterine serous papillary adenocarcinoma.

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MATERIALS AND METHODS

Establishment and HER-2/neu expression of USPC cell lines

Primary USPC tumor cell lines from 6 patients with invasive USPC were obtained from fresh tumor biopsies collected at the time of surgery, under approval of the institutional review board. Tumors were staged according to the International Federation of Gynecologists and Obstetricians operative staging system. Six primary USPC cell lines (USPC ARK-1, USPC ARK-2, USPC ARK-3, USPC ARK-4, USPC ARK-5, and USPC ARK-6) were established after sterile processing of the tumor samples from surgical biopsies as described previously.[15] Source-patient characteristics of these 6 USPC cell lines are described in Table 1. The amplification of the c-erbB2 gene by FISH, expression levels of HER-2/neu receptor by IHC, and messenger RNA expression levels by quantitative real-time polymerase chain reaction for these primary USPC cell lines have been recently reported[15] and are presented in Table 2.

Primary USPC growth rate analysis

The growth rate of each of the USPC cell lines was determined by counting the number of live cells 24, 48, 72 and 96 hours after plating. In brief, cell lines were cultured in RPMI 1640 (Mediatech, Manassas, VA) supplemented with 10% fetal bovine serum (Hyclone, Logan, UT). When cultures had grown to approximately 80% confluence, cells were harvested from the flask using 0.25% trypsin EDTA (Invitrogen, Carlsbad, CA), then counted on a hemacytometer chamber and assessed for viability via trypan blue exclusion. Cell density was adjusted to a concentration of 8000 cells/mL, then cells were seeded into a 384-well microtiter plate (Corning Life Sciences, Lowell, MA) at a density of 320 cells per well. Twenty-two replicate wells were plated per cell line per time point. Cell plates were incubated at 37°C with 5% carbon dioxide for 24, 48, 72, and 96 hours, at which time they were removed from the incubator, fixed with 95% ethanol (Fisher Scientific, Pittsburgh, PA) then stained with 4′,6-diamidino-2-phenylindole, dihydrochloride (DAPI) (Molecular Probes; Invitrogen, Carlsbad, CA). When DAPI staining was complete, cell plates were scanned on an automated fluorescent imaging system, and the number of cells in each well was counted.

Chemoresponse assay

The chemoresponse assay was performed as previously described.[16,17] The chemotherapy agents, concentrations, and combination used in the assays are described in Table 3. Briefly, cell lines were cultured in RPMI 1640 supplemented with 10% fetal bovine serum. When cultures had grown to approximately 80% confluence, cells were harvested from the flask using 0.25% trypsin EDTA, then counted on a hemacytometer chamber and assessed for viability via trypan blue exclusion. Cell density was adjusted to a concentration of 8000 cells/mL. Then cells were seeded into a 384-well microtiter plate at a density of 320 cells per well. Cell plates were incubated at 37°C with 5% carbon dioxide overnight. On the next day, serial dilutions of each anticancer agent or combination of 2 agents were prepared in growth medium to create 8, 9, or 10 distinct testing concentrations. In total, 14 single agents