Gynecologic Oncology 142 (2016) 76–82

Contents lists available at ScienceDirect

Gynecologic Oncology

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

Clinicopathologic characteristics and survival of patients with gynecologic malignancies metastatic to the brain

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HIGHLIGHTS

- Identification of poor outcome characteristics and long-term survival indicators
- Increased VEGF-A expression in metastatic brain samples compared to controls
- Multi-modality therapy was associated with improved clinical outcomes.

ARTICLE INFO

Article history:
Received 1 December 2015
Received in revised form 2 April 2016
Accepted 22 April 2016
Available online 8 May 2016

Keywords:
Gynecologic cancer
Brain metastases
Vascular endothelial growth factor
Ovarian cancer
Cervical cancer
Endometrial cancer

ABSTRACT

Objective. No standardized treatment strategies exist for patients with gynecologic malignancies complicated by brain metastases. Identification of poor outcome characteristics, long-term survival indicators, and molecular markers could help individualize and optimize treatment.

Methods. This retrospective cohort study included 100 gynecologic cancer patients with brain metastases treated at our institution between January 1990 and June 2009. Primary outcome was overall survival (OS) from time of diagnosis of brain metastases. We used univariate and multivariate analyses to evaluate associations between OS and clinical factors. We used immunohistochemistry to examine expression of five molecular markers in primary tumors and brain metastases in a subset of patients and matched controls. Statistical tests included the Student's paired *t*-test (for marker expression) and Kaplan-Meier test (for correlations).

Results. On univariate analysis, primary ovarian disease, CA-125 < 81 units/mL at brain metastases diagnosis, and isolated versus multi-focal metastases were all associated with longer survival. Isolated brain metastasis remained the only significant predictor on multivariate analysis (HR 2.66; CI 1.19–5.93; $p = 0.017$). Expression of vascular endothelial growth factor A (VEGF-A) was higher in metastatic brain samples than in primary tumors of controls ($p < 0.0001$). None of the molecular markers were significantly associated with survival.

Conclusions. Multi-modality therapy may lead to improved clinical outcomes, and VEGF therapy should be investigated in treatment of brain metastases.

1. Introduction

Metastatic brain lesions are uncommon in gynecologic malignancies; they occur in 0.3–0.9% of uterine corpus cancers [1–3], 0.3–11.6% of ovarian cancers [4–9], and 0.4–2.3% of cervical cancers [10–13]. Although rare, such lesions have become a focus of interest as advances in multimodality treatments are prolonging survival among gynecologic cancer patients, thereby allowing a larger percentage of patients to live long enough to develop distant metastases [4,14–17]. Such patients require evaluation for additional therapy options and present a new need for an extensive treatment risk/benefit analysis.

In most cases, the presence of metastatic brain lesions carries a grave prognosis. Previously, these patients were given supportive or palliative treatment, usually including chemotherapy, and were expected to survive only a few months. Improvements in both surgical technique and radiation therapy have led to additional options, and long-term survival

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http://dx.doi.org/10.1016/j.ygyno.2016.04.030
0090-8258/

is now possible in rare cases. Additionally, ovarian, endometrial, and cervical cancer patients in whom the brain is the first and only site of recurrent disease may achieve long-term benefits from treatment [3,7,9,18,19].

Molecular characteristics of tumors are becoming increasingly important in diagnosis, treatment, and prognosis of many malignancies. Immunohistochemistry (IHC) in gynecologic tumors and the subsequent brain metastases may identify a prognosticator and potentially targetable biomarker. We focused on five specific markers based on previous data and departmental experience: ephrin type-A receptor 2 (EphA2), estrogen receptor (ER), progesterone receptor (PR), multidrug resistance protein 1 (MDR1), and vascular endothelial growth factor A (VEGF-A).

EphA2 is a tyrosine kinase inhibitor involved in many cancer-related pathways including activation of focal adhesion kinase, suppression of integrin function, and activation of the extracellular signal-regulated kinases cascade. High levels of EphA2 correlate with aggressive features in ovarian carcinoma and brain metastasis in lung cancer [20,21]. Similarly, in endometrial and ovarian cancers, ER and PR status can be associated with adverse prognostic factors such as lymphovascular space invasion, and these receptors can serve as targets for treatment [22,23]. MDR1 is a permeability glycoprotein in the superfamily of ATP-binding cassette transporters. These receptors are responsible for decreased accumulation of drugs, such as the anticancer drugs doxorubicin and vinblastine, in multidrug-resistant cells. Consistent with its function at the blood-brain barrier, MDR1 is associated with increased risk of brain metastasis in ovarian cancer [22]. Lastly, VEGF-A has gained clinical relevance in treatment of gynecologic malignancies as we have seen success with the anti-angiogenic drug bevacizumab [24,25]. High expression of VEGF-A has also been associated with increased risk of central nervous system metastases in cancers that have a high propensity for brain metastases, such as non-small-cell lung cancer and melanoma [26,27].

Here, we sought to identify patient characteristics, disease features, and treatment modalities that associate with overall survival as well as evaluate expression of specific molecular markers with the hypothesis that they may be unique to gynecologic cancer patients with brain metastases.

2. Patients and methods

2.1. Study population

An Institutional Review Board-approved retrospective chart review included all patients with ovarian, fallopian tube, primary peritoneal, endometrial, cervical, vulvar, and vaginal cancers who were diagnosed with brain metastases between January 1, 1990 and June 30, 2009 at Washington University School of Medicine/Barnes-Jewish Hospital. Subjects were identified using ICD-9 codes for symptoms suggestive of brain metastases (seizures, altered mental status, other new onset neurological deficits, and hospice) to query the gynecologic oncology billing department. We included patients over age 18 with brain metastases originating from any gynecologic malignancy except gestational trophoblastic disease. Additional demographic and clinical data were extracted from both inpatient and outpatient paper and electronic medical records.

A central pathology review was conducted to confirm original cancer diagnosis. Brain metastases were confirmed by radiology reports, hospital charts, and pathology reports where possible. We obtained all available pathologic brain and primary tissue specimens of eligible subjects entered into the study. Brain biopsy specimens were available for only sixteen of the patients as many underwent biopsy at outside facilities or did not have remaining sample available for staining. We matched, at a 3:1 ratio, control patients within the same study period to brain samples from these 16 subjects (Fig. 1). Of the 16 patients for whom we had brain biopsies, we were able to obtain specimens from their primary malignancy in 5 cases. Control subjects were women diagnosed with gynecologic cancers with metastatic disease but who never experienced brain metastases. Patients were matched by type of cancer, age at time of original diagnosis, race, stage, and year of treatment.

2.2. Immunohistochemistry

Formalin-fixed, paraffin-embedded samples of primary tumors and brain metastases were stained as previously described [21,28–31]. Briefly, for EphA2, sections were deparaffinized and then probed with a monoclonal anti-EphA2 antibody (MedImmune, Gaithersburg, MD) overnight at 4 °C. Next, the slides were rinsed with phosphate-buffered saline-Tween 20, incubated with biotinylated linked antimouse IgG secondary antibody (Dako) for 30 min, incubated with a ready-to-use avidin-biotin complex method reagent (Dako) for 5 to 15 min, and then counterstained with Mayer hematoxylin (1:10) for 35 to 60 s. ER and PR immunostaining of paraffin sections was performed on a Ventana BenchMark ULTRA IHC Staining Module (Oro Valley, AZ) using prediluted anti-ER (SP1) and anti-PR (1E2) antibodies (FDA-approved method). MDR-1 staining was performed by using the Biogenex Super Sensitive Detection Kit (Biogenex Laboratories, San Ramon, CA) on the BioGenex i6000 Austostainer [30]. For VEGF-A, slides were incubated with rabbit polyclonal anti-VEGF antibody (1:50; Santa Cruz

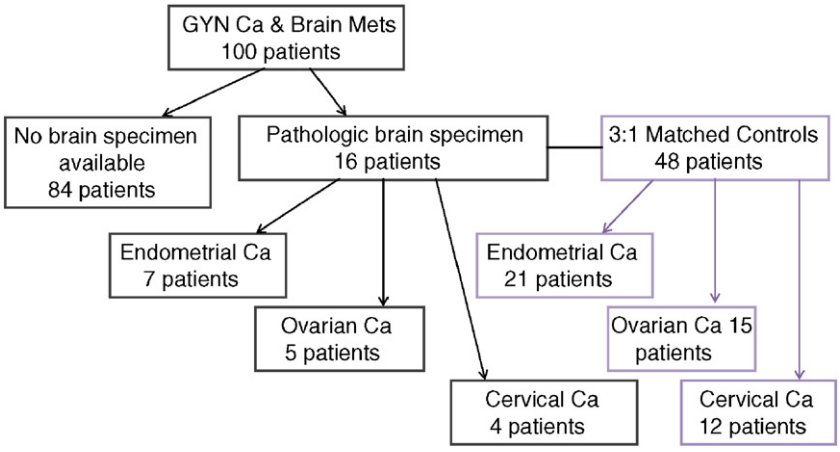

Fig. 1. 100 patients had brain metastases secondary to gynecologic cancer. For 84 patients there was no brain specimen available, 16 patients had pathologic brain tissue specimens available through our pathology department and were used for further molecular analyses. Of the 16 patients, 7 had endometrial cancer, 5 had ovarian, and 4 had cervical cancer. The sixteen cases of brain metastases were matched to 48 controls matched for type of cancer, age, race, stage, and year of treatment. For 5 of the 16 patients, matched brain and primary tissue samples were also available.