

Intraocular Metastases Secondary to Breast Carcinoma Correlates With Upregulation of Estrogen and Progesterone Receptor Expression in the Primary Tumor

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PURPOSE. To compare estrogen (ER), progesterone (PR), and human epidermal growth factor-2 (HER2) receptor expression in the primary tumor of patients affected by choroidal metastases from breast carcinoma (BC) versus those with extraocular metastases.

METHODS. Eighteen consecutive patients affected by choroidal metastases from BC were included. We defined ER, PR, and HER2 positivity of the primary tumor following standard guidelines. Breast carcinoma molecular subtypes were also identified (luminal A, luminal B, HER2-enriched, and triple negative). Forty consecutive patients affected by metastatic BC without choroidal involvement were included as a control group.

RESULTS. The study group and the control group were similar for age, sex, race, histopathologic classification of the primary tumor (ductal, lobular, others), and American Joint Committee on Cancer Tumor-Node-Metastasis stage at the time of primary tumor diagnosis ($P > 0.05$). Patients affected by choroidal metastases from BC showed a significantly higher expression of ER ($P = 0.009$) and PR ($P = 0.018$) receptors in the primary tumor compared with nonchoroidal metastatic BC. Across all patients, the luminal B molecular subtype was related to the presence of choroidal involvement ($P = 0.003$). Considering luminal tumors only, the luminal B subclassification was also related to the presence of choroidal involvement ($P = 0.009$).

CONCLUSIONS. Choroidal metastases from BC are associated with ER and PR expression in the primary tumor and the luminal B molecular subtype.

Keywords: metastases, breast, estrogen, progesterone, receptors

Uveal metastasis is the most common intraocular malignancy in the adult population, with a prevalence of 4% to 10% among carcinoma patients. The primary malignancy is usually located in the breast (47%) or in the lung (21%), and the choroid is the most common site of uveal metastases.¹⁻⁶ Most patients with choroidal metastases have already been diagnosed with systemic cancer at the time of eye tumor diagnosis, and choroidal metastases are generally associated with disseminated disease and poor prognosis.⁴ Nevertheless, in a consistent proportion (30%) of patients with choroidal metastases, the diagnosis of an intraocular secondary lesion precedes the diagnosis of the primary tumor.¹⁻⁶

About 1 in 8 women (12%) in the United States develop invasive breast carcinoma (BC) over the course of a lifetime. At present, BC is the most frequently diagnosed cancer with an increasing incidence worldwide and it is the leading cause of cancer-related deaths in women.⁷ Metastatic disease is reported to develop in up to 30% of node-negative (early stage) BC patients at diagnosis and in an even larger fraction of node-positive disease patients.⁸

Reliable, inexpensive tumor markers such as estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 (HER2) allowed the identification of the ER/PR/HER2 subtypes of BC, which is

the basis for current BC classification, prognostication, and therapy.⁹ Different subtypes of BC based on the ER/PR/HER2 classification have shown to have preferential site of metastases, adding increasing evidence of a complex, nonrandom pattern of distant metastatic spread in BC.⁸⁻¹⁰ The aim of the present study was to analyze the ER, PR, and HER2 receptor expression in the primary tumor of patients affected by choroidal metastases from BC, and to compare these data with a control population composed of metastatic BC patients without choroidal involvement.

PATIENTS AND METHODS

This was a retrospective observational case series with prospective enrollment, compliant with the tenets of the Declaration of Helsinki. The collaborative prospective ocular oncology database maintained at our ocular oncology unit was queried under institutional review board approval and informed consent was obtained from the subjects after explanation of the nature and possible consequences of the study. Patients were recruited from those referred between October 2011 and April 2016 to the ocular oncology service. Informed consent was obtained from each patient. Inclusion



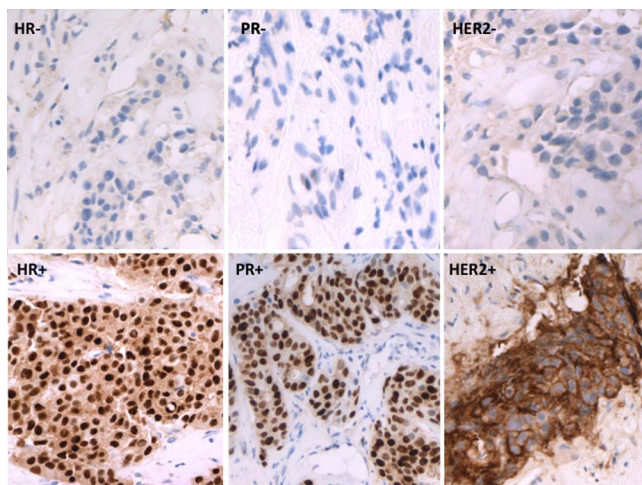


FIGURE. Immunohistochemical analysis of ER, PR, and HER2 expression in the primary tumor of patients enrolled in our tumor cohort. Representative positive (+) and negative (-) immunostaining for ER, PR, and HER2 expression ($\times 20$ magnification).

criteria were: patients aged 21 years or older and affected by choroidal metastases from histologically proven BC. Exclusion criteria were: lack of full original documentation of the primary tumor, including: the original histopathology classification (ductal, lobular, others); the tumor stage at diagnosis (American Joint Committee on Cancer Tumor-Node-Metastasis [AJCC-TNM])¹⁰; the receptor status (ER, PR, and HER2) of the primary tumor drawn from the formal histopathologic report and defined following standard guidelines.^{11–13} Briefly, receptor positivity (+) was defined as any positive nuclear staining (i.e., greater than or equal to 1%) for ER and PR and an immunohistochemistry score of 3+ or immunohistochemistry score of 2+ plus fluorescent in situ hybridization with amplification ratio ≥ 2.0 for HER2 (Figure). Intrinsic breast cancer subtypes were determined according to criteria recently recommended by the St. Gallen International Breast Cancer Conference. The following definitions were used: luminal A (ER and/or PR positive, HER2 negative, Ki67 inferior to 14%); luminal B (ER and/or PR positive, Ki67 $\geq 14\%$, and/or HER2 positive); HER2-enriched (ER and PR negative, HER2 positive, and any Ki67); and triple negative (ER and PR negative, HER2 negative, and any Ki67).¹³

All patients underwent full ophthalmic examination at baseline, including color fundus photography, A/B-scan ultrasonography (US), and spectral-domain optical coherence tomography (SD-OCT) using a commercial device (Spectralis; Heidelberg Engineering, Heidelberg, Germany). The clinical diagnosis of choroidal metastasis from breast carcinoma was confirmed in each case by a single experienced ocular oncologist.

Forty consecutive patients affected by metastatic BC without choroidal involvement, referred to our clinic for routine ophthalmologic procedures (such as outpatient diagnostic procedures or treatments), were also enrolled as a control group. The absence of choroidal involvement was confirmed in each case by the same experienced ocular oncologist.

The statistical analysis was carried out according to the usual methods of descriptive statistics: frequency distribution and percentages. Demographic and clinical data were also described in terms of mean, standard deviation, and range (minimum-maximum). Categorical variables were compared using the χ^2 test or Fisher's exact test, as appropriate; continuous variables were compared using the Student's *t*-test

or the Wilcoxon Mann-Whitney test as appropriate. All analyses were performed using statistical software (SAS, version 9.3; SAS Institute, Cary, NC, USA).

RESULTS

Eighteen consecutive patients affected by choroidal metastasis from histologically proven BC were included (mean age, 54.1 ± 11.1 years; range, 38–84). Seventeen patients were female (94.4%) and a single patient was male (5.6%). Uveal metastases were unifocal in 8 (44.4%) and multifocal in 10 (56.6%), with unilateral involvement in 12 patients (66.7%) and bilateral involvement in 6 patients (33.3%). Ultrasound examination revealed a medium internal reflectivity with irregular structure in 15 cases (83.3%). Perilesional serous retinal detachment was detectable by US in 9 cases (50.0%) and in 18 cases (100%) by SD-OCT. Anterior compression/obliteration of the overlying choriocapillaris and an irregular (lumpy-bumpy) anterior contour was also detected by SD-OCT in 12 (66.7%) and 14 tumors (77.8%), respectively. All uveal metastases were choroidal in location. By histopathologic classification, the primary tumor was invasive ductal carcinoma in 16 cases (88.9%) and invasive lobular carcinoma in 2 cases (11.1%). At the time of primary tumor diagnosis, AJCC-TNM classification was stage I in five patients (27.8%), stage II in seven patients (38.9%), stage III in four patients (22.2%), and stage IV in two patients (11.1%).

The study group and the control group were similar in age, sex, race, histopathologic classification of the primary tumor (ductal, lobular, others) and AJCC-TNM stage at the time of primary tumor diagnosis ($P > 0.05$; Table).¹⁰

Patients affected by choroidal metastases from BC were characterized as ER+ in 18 cases (100%), PR+ in 16 cases (88.8%), and HER2+ in 3 cases (16.7%). Patients affected by metastatic BC without choroidal involvement were characterized by ER+ in 28 cases (70%), PR+ in 23 cases (57.5%), and HER2+ in 8 cases (20%). Patients affected by choroidal metastases of BC showed a statistically significant higher expression of ER ($P = 0.009$) and PR ($P = 0.018$) receptor in the primary tumor compared with nonchoroidal metastatic BC.

Following the St. Gallen International Breast Cancer Conference surrogate definition of intrinsic subtypes of BC, all patients (100%) affected by choroidal metastases of BC had luminal tumors. Of these, 5 patients were affected by luminal A (27.8%) and 13 by luminal B (72.2%) BC. No patients were affected by HER2-enriched or triple negative BC molecular subtypes. In the control group, 23 patients (57.5%) were affected by luminal A, 12 by luminal B (30%), 2 patients (5%) by HER2-enriched, and 3 patients (7.5%) by triple negative BC molecular subtypes. Considering all patients, the luminal B molecular subtype was associated with choroidal involvement ($P = 0.003$). Considering luminal tumors only, the subclassification in luminal B was also associated with choroidal involvement ($P = 0.009$).

DISCUSSION

Uveal metastasis from carcinoma represents an increasing problem in the context of an aging population and enhanced survival of stage IV cancer patients.^{1–4} These lesions may progress rapidly and are potentially sight-threatening.⁴ Early diagnosis and appropriate timely treatment are therefore mandatory to maintain patients' quality of life. External beam radiotherapy (EBRT), chemotherapy, hormone and biologic therapies, brachytherapy, and photodynamic therapy are the main therapeutic modalities for the management of these lesions, with the strongest evidence supporting timely EBRT

TABLE. Clinical and Demographic Characteristics of Enrolled Patients

	Study Group*	Control Group†	P Value	All Patients
Patients, <i>n</i>	18	40	–	58
Age, y, mean (range)	54.1 ± 11.1 (39–84)	55.9 ± 10.7 (38–83)	>0.05	55.2 ± 11.2 (38–84)
Sex, female/male (%)	17/1 (94/6)	40/0 (100/0)	>0.05	57/1 (98/2)
Race, Caucasian/other races (%)	17/1 (94/6)	38/2 (95/5)	>0.05	55/3 (95/5)
Affected breast, right/left/both (%)	9/8/1 (50/44/6)	19/18/3 (47/45/7)	>0.05	28/26/4 (48/45/7)
Primary tumor histology, ductal/lobular/others (%)	16/2/0 (89/11/0)	36/4/0 (90/10/0)	>0.05	52/6/0 (90/10/0)
Affected eye, right/left/both (%)	7/5/6 (39/28/33)	NA	–	NA
AJCC-TNM ¹⁰ stage at the time of primary tumor diagnosis				
Stage I, <i>n</i> (%)	5 (28)	14 (35)	>0.05	19 (33)
Stage II, <i>n</i> (%)	7 (39)	15 (37)	>0.05	22 (38)
Stage III, <i>n</i> (%)	4 (22)	6 (15)	>0.05	10 (17)
Stage IV, <i>n</i> (%)	2 (11)	5 (13)	>0.05	7 (12)
Receptor status (primary tumor)				
ER+, <i>n</i> (%)	18 (100)	28 (70)	0.009‡	46 (79)
PR+, <i>n</i> (%)	16 (89)	23 (57)	0.018‡	39 (67)
HER2+, <i>n</i> (%)	3 (17)	8 (20)	>0.05	11 (19)
Molecular subtypes (primary tumor)				
Luminal A, <i>n</i> (%)	5 (28)	23 (57)	0.036‡	28 (48)
Luminal B, <i>n</i> (%)	13 (72)	12 (30)	0.003‡	25 (43)
HER2-enriched, <i>n</i> (%)	0 (0)	2 (5)	>0.05	2 (3)
Triple negative, <i>n</i> (%)	0 (0)	3 (7)	>0.05	3 (5)

NA, not applicable.

* Patients affected by choroidal metastases from BC.

† Patients affected by metastatic BC without choroidal involvement.

‡ Statistically significant.

(grade B; strong support for recommendation).¹⁴ Nevertheless, alternative or adjuvant therapies, such as systemic chemotherapy and hormone therapies for choroidal metastases from BC, are recently emerging.^{3,6,15,16} Demirci et al.⁶ reported a local tumor control rate of 81% using systemic chemotherapy or hormone therapy in affected patients. Thus, a more deep knowledge of the ER/PR/HER2 receptor status of patients with choroidal metastatic involvement by BC could potentially influence the treatment of these patients.

Major BC intrinsic molecular subtypes are defined by the expression of hormone receptors and HER2, with or without an accompanying Ki-67 proliferation index. The resulting regulation of different signalling pathways and gene expression profiles influences the distinct biologic behavior of these tumor subtypes, including aggressiveness and organ-specific metastatic potential.^{17–20}

Expression of ER and PR receptors by choroidal metastases of BC and the consequently good response to hormonal therapy have been previously reported in single case reports.^{3,15,16} Nevertheless, no work focusing on the ER, PR, and HER2 receptor expression in the primary tumor of patients affected by choroidal metastases of BC has been published, nor have there been any reports investigating the intraocular specific metastatic potential of different BC molecular subtypes.

In our prospectively collected series, all patients with choroidal metastases of BC showed ER positivity and, in a high percentage of them, also PR positivity. Moreover, combining the receptor status of patients affected by choroidal metastases by BC, 76% of these patients have a primary tumor classified as luminal B. Compared with population-based studies, the luminal B phenotype is expected to be present in a much lower proportion (20% to 30% of BC overall).^{9,13} This difference is highlighted also by the luminal B percentage of our control group (30%).^{9,13,17} The presence of receptor

positivity in each primary BC with choroidal metastases is of clinical importance, both from biologic and therapeutic perspectives.

Luminal tumors have cells resembling those of tumors starting in the inner (luminal) cells lining the mammary ducts. Patients with luminal B tumors are diagnosed at a younger age than those with luminal A tumors. Compared with luminal A tumors, luminal B tumors tend to have factors that lead to a relatively poorer prognosis, including higher tumor grade, larger tumor size, and lymph node positivity at diagnosis.^{10,20} Some (about 30%) luminal B tumors contain p53 gene mutations. Women with luminal B tumors, compared with HER2-enriched and triple negative tumors, tend to have fairly high survival rates, although not as high as those with luminal A tumors.^{10,20}

Among all BC molecular subtypes, the skeleton was the most common site of metastatic disease, representing approximately half (48%) of patients, followed by liver (27%), lung (23%), central nervous system (17%), and pleura (7%).²⁰ Breast cancer subtypes differ not only in primary tumor characteristics, but also in their metastatic behavior. This fact could potentially be used in determining the appropriate strategy for patient follow-up. The luminal A and B subtypes are associated with more bone metastases compared with the HER2-enriched and triple negative BC.²⁰ Furthermore, the luminal A subtype is associated with bone only metastases. Liver metastases are more frequently observed in HER2-enriched compared with luminal A and triple negative BC, and the luminal subtypes are less frequently associated with lung metastases compared with triple negative BC.²⁰ The luminal A subtype is also less frequently associated with central nervous system metastases than HER2-enriched and triple negative BC. Similarly, the luminal B subtype is less frequently associated with central nervous system metastases compared with the HER2-enriched,

showing a similar trend when compared with triple negative BC.²⁰

Soni et al.¹⁸ recently reported that luminal tumors are remarkable for their significant bone-seeking phenotype and are less frequently observed in lung, brain, and pleural metastases, and less likely to be associated with multiorgan relapse. Notably, these authors reported that there was no significant difference between the luminal A and B subtypes for any site of relapse.¹⁸ In the present study, a statistically significant association of metastatic homing to the choroid was observed for luminal B breast cancer, suggesting that this particular subtype of BC may have the choroid as a preferential homing. The mechanisms by which the luminal B breast cancer tumors tend to metastasize to the choroid remain to be elucidated. Thus, further investigations into the molecular mechanisms of this relationship are warranted.

The higher expression levels of ER and PR in the primary tumor of patients affected by metastatic BC with choroidal involvement raises the possibility that this knowledge could potentially be used in determining the appropriate treatment for these patients, such as the use of the aromatase inhibitors. However, the major limitation of this study is that BC receptors status was not analyzed directly in the intraocular lesion, but only in the primary tumor. It would be certainly interesting to know whether such increase in ER and PR expression is maintained in the secondary lesion or whether the choroidal metastases have different expression levels of these receptors, possibly due to a different microenvironment compared with the tissues surrounding the primary tumor. Unfortunately, given that intraocular metastases are mostly treated by conservative (nonsurgical) treatment, no material is available to perform these analyses directly in the choroidal lesions.⁶ Moreover, at least in the standard clinical practice, patient treatment is planned assuming, in absence of other direct evidence, that both primary tumor and metastases have similar receptors status.⁸

Another possible limitation of this study is that the primary tumor of most of the enrolled patients was diagnosed in local referral centers. Therefore, the specimens of the primary tumors were not fully available for additional assays to confirm the data obtained by standard immunohistochemistry. Lack of standardization, technical variability in laboratory protocols, and subjective interpretation are major problems associated with immunohistochemistry; therefore, these results need future confirmation by quantitative PCR or Western blot.

Choroidal metastases are often considered by nonophthalmologists as a group with other central nervous system metastases, which are reported to have a greater incidence in nonluminal BC.⁹ Our results underline that BC with choroidal involvement is most often luminal B breast cancer, and thus biologically different from those that preferentially metastasize to the central nervous system. These findings hint that a clear distinction has to be made, given the different histologic nature of the choroid, which is primarily a vascular structure, from nervous tissue, as well as the difference in expected molecular BC subtypes homing to the choroid and the central nervous system.

In conclusion, choroidal metastases by BC were associated with ER and PR expression in the primary tumor and the luminal B molecular subtype. Despite the small sample size of our population, these results suggest that primary tumor receptor expression and molecular subtype may influence the choroidal metastatic tropism of BC. Characterization of BC subtypes and their metastatic behavior could improve the diagnostic and screening approach to patients affected by this tumor. The identification of a specific BC molecular subtype associated with choroidal involvement could also prevent the worsening of debilitating vision-related symptoms with prompt

systemic treatment based on the expected sensitivity to antihormone drugs.

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