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Gynecologic melanomas: A clinicopathologic and molecular analysis☆

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HIGHLIGHTS

- A subset of gynecologic melanomas (18.7%) involves the vagina and/or cervix.
- Non-vulvar melanomas show high-risk clinicopathologic features.
- The long-term clinical outcome of non-vulvar melanomas is poor.
- *BRAF*, *KIT*, *NRAS*, and *CTNNB1* mutations are uncommon in gynecologic melanomas.
- The majority of gynecologic melanomas do not harbor targetable oncogenic mutations.

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ABSTRACT

Objective. Melanoma originating from gynecologic sites (MOGS), including the vulva, vagina, and cervix, is a rare and aggressive form of melanoma with poor long-term clinical outcome. The clinicopathologic features of vulvar and non-vulvar tumors remain relatively understudied, and in contrast to cutaneous melanomas at non-sun-exposed sites, MOGS typically do not harbor *BRAF* mutations. Thus, we sought to analyze the clinicopathologic and molecular features of MOGS.

Methods. A large retrospective cohort of patients with MOGS ($n = 59$) at a single large academic institution over a 28-year period was identified. Associations among clinicopathologic characteristics were assessed via standard statistical approaches, and clinical outcome was examined using Cox regression analysis. Sanger sequencing was utilized to identify mutations in hotspot regions of *BRAF*, *KIT*, *NRAS*, and *CTNNB1*.

Results. Tumors involving the vagina and/or cervix (non-vulvar) are significantly associated with high-risk clinicopathologic features, including increased tumor thickness, ulceration, positive resection margins, lymph node metastasis, and poor long-term clinical outcome (with increased risk of death due to disease). The aggressive clinical behavior of non-vulvar tumors is independent of advanced clinical stage and lymph node metastasis in multivariate analysis. Targeted molecular analysis confirms an overall low rate of oncogenic mutations in our MOGS cohort, although *KIT* mutations (particularly in exon 11) are relatively enriched.

Conclusions. Overall, our results show that non-vulvar MOGS are aggressive tumors with poor long-term clinical outcome and indicate that few targeted therapeutic options are currently available to patients with MOGS.

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1. Introduction

Malignant melanoma is an increasingly common diagnosis, with incidence rates that have been on the rise globally for decades [1,2]. These tumors most often occur on the skin at both sun-exposed and non-sun-exposed sites, less commonly arise from mucosal surfaces, and rarely develop within internal organs [1]. Melanoma originating from gynecologic sites (MOGS), including the vulva, vagina, and cervix, is rare, accounting for only 1–3% of melanoma in women [3–7]. According to a retrospective analysis of >80,000 cutaneous and non-cutaneous melanomas from the National Cancer Data Base, only 1.3% of tumors arise from mucosal sites, and of these, <20% occur in the female genital tract [1].

MOGS is associated with a poor clinical outcome and may have the worst prognosis among all melanoma subtypes, with reported five-year survival rates ranging from 10 to 47% [1,3,5,6,8]. The aggressive clinical behavior of MOGS is likely multifactorial, including lack of accepted screening methods and advanced clinical stage at presentation [9–11]. Although currently there are no consensus guidelines regarding clinical management of MOGS, patients with clinically localized disease typically undergo primary surgical excision, with or without sentinel lymph node biopsy and/or regional lymph node dissection. For patients with locally advanced disease, more radical surgical approaches may not be indicated, as several studies have demonstrated increased morbidity without a significant survival benefit [9–11]. Like other melanomas, MOGS does not typically respond to conventional chemotherapy or radiation therapy, which limits therapeutic options for patients with metastatic disease [9–11]. Recent molecular advances have identified recurrently mutated oncogenes, including *BRAF*, *NRAS*, and *KIT*, in a variety of melanoma subtypes, spurring the development of targeted therapeutics for potential clinical use [12]. Studies of recurrent molecular alterations in MOGS have been relatively limited [13–24]; while a subset of tumors harbors *KIT* mutations, MOGS does not appear to have a high frequency of *BRAF* or *NRAS* mutations.

Overall, few studies have reported both detailed clinicopathologic analysis with long-term clinical outcome data and molecular results for MOGS [13–15]. Therefore, in this study, we undertook a retrospective clinicopathologic review and molecular analysis of a large retrospective cohort of MOGS treated at a single large academic institution over a 28-year period with long-term clinical follow-up.

2. Materials and methods

This study was approved by the University of Michigan Medical School Institutional Review Board. All MOGS cases between 1985 and 2013 were retrospectively identified from the surgical pathology records database of Michigan Medicine using exhaustive keyword searches; additional cases were identified using pre-existing patient lists obtained from the multidisciplinary melanoma clinic. Only patients with primary MOGS were included, and the possibility of metastasis from a non-gynecologic primary site was excluded on clinicoradiographic grounds. For all cases, available slides were reviewed by an experienced dermatopathologist (R.M.P.) to confirm the diagnosis. Standard histopathologic parameters, including tumor thickness (Breslow depth), histologic subtype, presence of ulceration, mitotic rate, presence of associated nevus, pathologic margin status after resection, and lymph node status, were extracted from the associated pathology report. Pathologic staging and clinical staging was determined using the American Joint Committee on Cancer (AJCC) 7th Edition Cancer Staging System [25]. For the purposes of this study, vulvar tumors were defined as those that clinically and pathologically only involved the vulvar mucosa, without concomitant involvement of the vaginal and/or cervical mucosa; in contrast, non-vulvar tumors were defined as those that clinically and pathologically involved the vaginal and/or cervical mucosa, regardless of concomitant involvement of the vulvar mucosa. Clinical information, including age at diagnosis, race/ethnicity, location of tumor, length of follow-up, recurrence, and death due to disease, was obtained by manual review of the electronic medical records database.

Cases with adequate tissue for molecular analysis were identified by retrospective review of available slides, and areas of representative primary tumor were specified by an experienced dermatopathologist (R.M.P.). For each case, tumor DNA was extracted from sectioned formalin-fixed paraffin-embedded (FFPE) tissue using the Pinpoint Slide DNA Isolation System (Zymo Research, Irvine, CA), according to the manufacturer's instructions. Tumor had to comprise at least 30% of macrodissected tissue, corresponding to current clinical standards for Sanger sequencing at our institution. Standard PCR with custom primers was utilized to amplify genomic regions of interest in *BRAF* (exon 15), *NRAS* (exons 2 and 3), *CTNNB1* (exon 2), and *KIT* (exons 9, 11, 13, 17, and 18) (see Supplemental Table 1 for details). After confirming the expected amplicon size by standard agarose gel electrophoresis, all PCR products were subjected to bi-directional Sanger sequencing by the University of Michigan DNA Sequencing Core using custom primers. The resulting chromatograms were examined manually, and the significance of detected mutations was evaluated manually using the Catalogue Of Somatic Mutations In Cancer (COSMIC) and single nucleotide polymorphism (SNP) databases, including dbSNP and ExAC.

All statistical analyses were performed in Excel (Microsoft, Redmond, WA) using the XLSTAT package (Addinsoft SARL, Paris, France). Fisher's exact and Chi-squared tests were utilized to examine associations among qualitative (categorical) data, while relationships among quantitative (numerical) data were explored using parametric (Student's t) and non-parametric (Mann-Whitney) tests, as indicated. For outcome analysis, survival time was defined as time from date of initial surgical resection to the sooner of: 1) death due to disease; or, 2) last follow-up. Univariate and multivariate Cox proportional hazard ratios were utilized to examine the association between clinicopathologic parameters and time to death due to disease.

3. Results

Overall, 59 patients with MOGS were identified for inclusion in this study (see Table 1 for cohort details). These patients tended to be older (median age at diagnosis = 53 years; range = 10–94 years), and the vast majority were Caucasian ($n = 56$; 94.9%). Most of the tumors occurred exclusively on the vulva ($n = 48$; 81.3%), with a smaller subset involving both the vulva and vagina ($n = 6$; 10.2%) or vagina and/or cervix ($n = 5$; 8.5%). Fourteen (23.7%) tumors were in situ melanoma and the remaining ($n = 45$; 76.3%) were invasive. The median tumor thickness was 1.1 mm (range = 0–85.0 mm). The most common histologic subtype was superficial spreading ($n = 18$; 30.5%). A preexisting nevus was identified in 14 (23.7%) patients, and ulceration was present in 15 (33.3%) invasive tumors. The mitotic rate ranged from 0 to 31/mm^2 (median = 2). The majority of patients ($n = 42$; 71.2%) either had negative histologic margins after primary surgical resection or had a positive margin cleared on subsequent re-resection. A small subset of patients ($n = 7$; 11.8%) had confirmed lymph node metastasis at the time of primary resection. Sentinel lymph node biopsy was performed in 21 (35.6%) patients, and three (14.3%) of these patients had at least one positive sentinel lymph node. One patient subsequently had a negative inguinal lymph node dissection, while the two other patients did not undergo completion lymphadenectomy. A regional lymph node dissection was performed in 11 (18.6%) patients. Four (36.4%) of these patients underwent a superficial inguinal lymph node dissection, with a single large nodal metastasis identified in one patient. A superficial and deep groin dissection was performed in the other 7 (63.6%) patients, and three (42.8%) of these patients had confirmed regional disease in the pelvic lymph node basin. Overall, 32 (54.2%) patients were either clinical stage 0, IA, or IB at presentation, and no patients presented with stage IV (i.e., distant metastatic) disease. Clinical follow-up ranged from 0.5–297 months (median = 49 months).