Vulvar and vaginal melanoma: A distinct subclass of melanoma based on a comprehensive molecular analysis of 51 cases

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Abstract

Objectives:
Vulvar melanomas are the second most common vulvar malignancies. These rare cancers are biologically aggressive, and may harbor distinct molecular characteristics from cutaneous and mucosal melanoma of other sites. We analyzed and compared molecular, genomic and protein expression patterns in vulvar/vaginal melanoma (VM) to a large cohort of melanoma of non-gynecological (NGM) origin.

Methods:
2304 cases of melanoma were submitted for molecular profiling from 2009 to 2015. In situ hybridization and immunohistochemistry were used to assess copy numbers and protein expression respectively, of selected genes.

Results:
Out of 51 cases of malignant VM, 14 were of vaginal and, 37 were of vulvar origin. Table 1 summarizes the characteristics of the analyzed cases. All tumors were analyzed using Illumina TruSeq Amplicon Cancer panel to search for sequence variants in genes commonly implicated in carcinogenesis. We also analyzed the frequency of biomarkers of interest in VM, and NGM which we further classified into cutaneous, acral and mucosal based on site of origin. BRAF is most frequently mutated in VM (26%), compared to 36.6% in cutaneous melanoma, and 8.3% (p=0.008) in mucosal melanoma. However, BRAF mutations in VM are significantly less likely to include known responders to BRAF inhibitors than those from NGM tumors (p=0.011). c-KIT mutation rate in VM (22%) is significantly higher than in cutaneous (3%, p<0.001) and mucosal (8.8%, p=0.05) melanoma. The majority (60%) of cKIT mutations in VM are also known to be sensitive to inhibitors of tyrosine kinase receptor. NRAS mutations are rare in VM (4%), compared to cutaneous (25.9%, p=0.009), and acral (40.6%, p=0.002) melanoma. VM express biomarkers of cytotoxic sensitivity more commonly than NGM, including increased TOP2A and RRM1, which are markers of anthracycline and gemcitabine resistance, respectively (p=0.0001, 0.006). PDL1 is expressed frequently in both VM and NGM (56%, 63.5%), PI3KCA mutations, and ER/PR receptor expression are rare.

Conclusions:
Our findings suggest VM may represent a unique subclass of melanoma. VM are unlikely to harbor mutations sensitive to existing BRAF inhibitors. Tyrosine kinase inhibitors or MEK inhibitors targeting c-KIT and NRAS gene may be of therapeutic benefit. PDL1 inhibitors warrant further exploration in patients with melanoma from gynecological tract.

Results

Table 1. Characteristics of the 2,304 cases of melanoma submitted for molecular profiling from 2009 to 2015

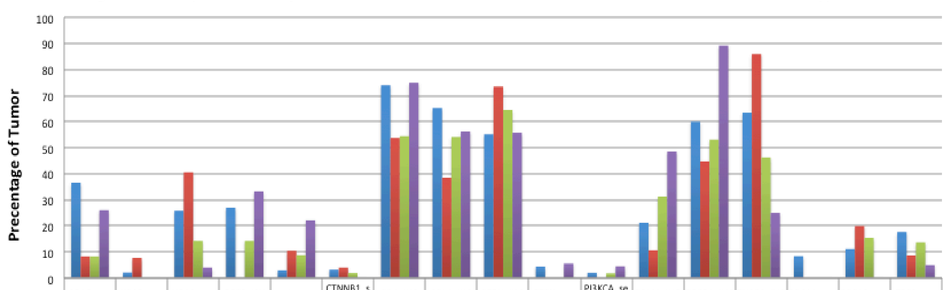
Mel-subtypes	% Metastatic	Age Groups (years)			Gender	
		19-39	40-65	66-97	M	F
Gyn (n=51)	41% (21/51)	8% (4/51)	33% (17/51)	59% (30/51)	0% (0/51)	100% (51/51)
Cutaneous (n=1975)	53% (1038/1975)	8% (163/1975)	46% (908/1975)	46% (904/1975)	64% (1264/1975)	36% (711/1975)
Acral (n=21)	24% (5/21)	0% (0/21)	57% (12/21)	43% (9/21)	43% (9/21)	57% (12/21)
Mucosal (n=105)	56% (59/105)	2% (2/105)	53% (56/105)	45% (47/105)	45% (47/105)	55% (58/105)

Table 2. Mutational frequency distribution between VM and NGM

Mutation	VM (%)	NGM (%)
v600E	50%	66%
G469A	16.70%	0.60%
D594N	8.30%	
D594E	8.30%	
D594H		
T599_V600del		

•The mean age of diagnosis for NGM was 63 and the mean for VM was 65 years of age
•BRAF was mutated in 34.2% of the entire melanoma cohort. 26% of VM harbored BRAF mutation, compared to 36.6% in cutaneous, and 8.3% of mucosal and acral NGM group (p=0.008).
•The most common BRAF mutations found in VM are distinct from NGM tumors. Table 2 shows distinct mutation profiles between VM and NGM in the BRAF gene.

Figure 1. Comparative Frequencies of Alteration in Biomarker of Interest Across Melanoma Subtypes



	BRAF_seq	KRAS_seq	NRAS_seq	EGFR_ISH	c-kit _seq	CTNNB1_seq	PD1_IHC	PDL1_IHC	PTEN_IHC	PTEN_seq	PI3KCA_seq	RRM1_IHC	TOP2A_IHC	TUBB3_IHC	BRCA1_seq	BRCA2_seq	TP53_seq
Cutaneous	36.6	2.1	25.9	27	3	3.3	74	65.3	55.2	4.4	2	21.2	60	63.5	8.4	11.2	17.7
Acral	8.3	7.7	40.6	0	10.5	4	53.8	38.5	73.5	0	0	10.7	44.8	86	0	20	8.7
Mucosal	8.3	0	14.3	14.3	8.8	1.9	54.5	54.2	64.5	0	1.8	31.3	53.1	46.3	0	15.4	13.7
Gyn	26.1	0	4	33.3	22.2	0	75	56.3	55.8	5.6	4.5	48.6	89.2	25	0	0	5

Background

- Gynecological malignant melanomas are biologically aggressive cancers Understanding genetic alterations of cutaneous melanoma has allowed for the development of pharmacological inhibitors . The genetic alterations most commonly seen in non-gynecological malignant melanomas involve the NRAS and BRAF codons both of which are involved in the MAP kinase pathway
- Activating mutations in BRAF are present in 50% of advanced non-gynecological melanomas whereas 20% of malignant melanomas have activation of NRAS
- Although targeting specific mutations provides a promising adjuvant treatment option in advanced melanoma, the incidence and variation of genetic mutations is not well established in gynecological melanoma

Results cont'd

•c-KIT mutation rate in the general melanoma cohort is 4.%, and is significantly higher in VM (22%), compared to NGM (3.5%) (p=0.0001). Within the NGM cohort, acral melanoma had the highest c-KIT mutation rate
•60% of cKIT mutations seen in VM were reported to be sensitive to Receptor Tyrosine Kinase inhibitors
•VM express biomarkers of cytotoxic sensitivity more commonly than NGM, including increased TOP2A and RRM1, markers of anthracycline and gemcitabine resistance, respectively (p=0.0001, 0.006)
•TUBB3, a marker of resistance to taxanes, was expressed in 25% of VMs compared to 63.5% of cutaneous NGMs and 86% of acral melanomas
•PDL1 is expressed frequently in both VM and NGM (56%, 63.5%), mutations, and ER/PR receptor expression are rare overall.

Conclusions

- VM may represent a unique subclass of melanoma
- Regarding BRAF, less mutations were seen in VM compared to NGM and of the BRAF mutations seen only 50% contained V600E mutations
- BRAF mutations in VM may not be sensitive to existing inhibitors
- Tyrosine kinase inhibitors or MEK inhibitors targeting c-KIT and NRAS gene may be of therapeutic benefit.