Intratumoral heterogeneity in melanoma

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Introduction & Objectives: Cutaneous melanoma is associated with unpredictable prognosis, because of resistance to conventional antineoplastic treatments. It has been suggested that intratumoral heterogeneity may be responsible for treatment resistance in some solid tumors. Intratumoral heterogeneity is defined as the existence of diverse tumor clones with different microscopic and genetic features within a single neoplasm and its metastases. We aim to assess intratumoral heterogeneity in primary superficial spread (SSM) and nodular (NM) cutaneous melanomas and their paired metastases, by assessing differences in microscopic appearance and mutational burden of several areas of these tumors.

Materials & Methods

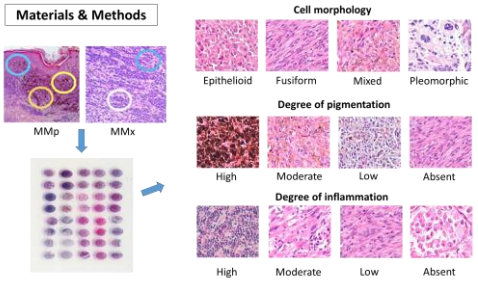

Figure 1) A retrospective review of our Pathology and Molecular Genetics Department archive between 2000-2019 has been carried out. Selection of paired primary tumor (MMp) and its metastases (MMx) from SSM and NM subtypes was performed. A detailed microscopic analysis of different tumor characteristics such as areas of different cell morphology or degree of pigmentation has been conducted, selecting 3 or 2 regions with different characteristics in the MMp and the MMx, respectively. Areas selected were microdissected for a Tissue Microarray construction and DNA extraction.

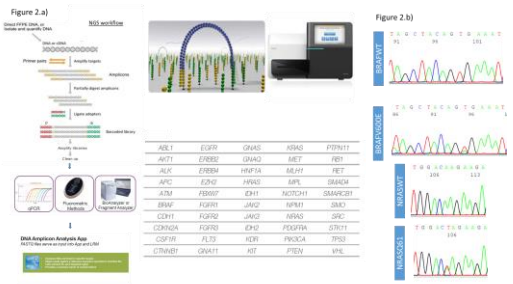

Figure 2. a) Genetic characterization of MMp and MMx paired areas by next generation sequencing (NGS) with a Cancer Hotspot panel that studies 2800 COSMIC mutations from 50 oncogenes and tumor suppressor genes with known cancer associations. Areas were the same used for the TMA construction **b)** Presence of BRAF and NRAS mutations were verified by SANGER sequencing.

Results

GENDER	10M/4F (14 patients)
AGE (years)	62 (24-86)
LOCATION OF MMp	6 Body, 4 Lower limbs, 2 Upper limbs, 2 Head/neck
MM subtype	9SSM/5NM
BRESLOW (mean in mm)	3,77 (1,04-6,23)
CLARK	4(3-5)
MITOSES (mean of mitoses/mm²)	4,15 (1-10)
ULCERATION	9/14 (64,28%)
TOTAL Nº OF MMx	47(3,36/patient)
MMx LOCATION	19 skin, 14 nodal, 4 liver, 4 CNS, 3 lung, 2 parotid, 1 bone
DEBUT MMx Nº	15 (1,07/patient)
DESEASE FREE SURVIVAL (months)	16,78 (0-123)
DEAD	8/14

Table 1: Descriptive analysis of our cohort of 12 patients.

GENE	MUTATION	CONSEQUENCE	Nº CASES
BRAF	V600E	Missense	11/14 (79%)
NRAS	R68T, Q61L, Q61K	Missense	3/14(21%)
KDR	Q472H	Missense	8/14 (14%)
TP53	P72R, G108S, R213*, N/A	Missense, Syn. variant, Stop	14/14 (100%)
KIT	P34L, M541L	Missense	4/14 (29%)
CDKN2A	P114L, P81L, R80*	Missense, Stop	3/14 (21%)
PIK3CA	I391M	Missense	3/14 (21%)
PDGFRA	NA	Syn. variant	14/14 (100%)
FGFR3	F384L, N/A	Missense, NA	14/14 (100%)

N/A: Not available

Table 2: NGS of 41 MMp and 27 MMx paired regions was performed. Data were filtered to exclude all synonymous and intronic variants. A quality score cutoff of 100 and an allele frequency > 15%, with a sequencing coverage >150X were also applied to eliminate low frequency artefacts. 26 mutations were identified (19 missense (73%) ; 1 unknown/NA (4%); 3 stop (11%), 2 synonymous variant (8%) and 1 deletion (4%)). All mutations present in MMp were also present in their paired MMx. Mutations present in ≥2 cases are shown. Other mutations found were in ERBB4, PTEN, ATM, MET, SMO and STK11 genes.

High frequency of BRAFV600E mutation correlate with spindle cell phenotype and high inflamatory infiltrate

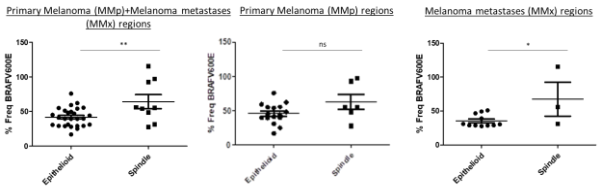

Figure 3) T-test analysis of MMp+MMx, MMp and MMx groups showed that spindle cell regions had significant higher frequency of BRAFV600E mutations in MMp+MMx and MMx groups (p=0.0039; p=0.0741 p=0.0275)

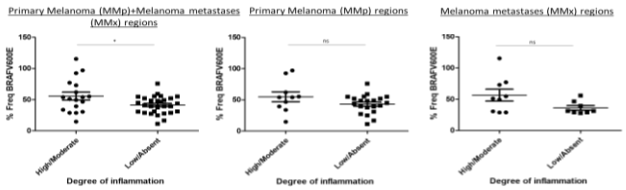

Figure 4) ANOVA test of MMp+MMx, MMp and MMx groups showed that moderate/severe inflammatory infiltrate had significant higher frequency of BRAFV600E mutations in MMp+MMx group (*p<0,05).

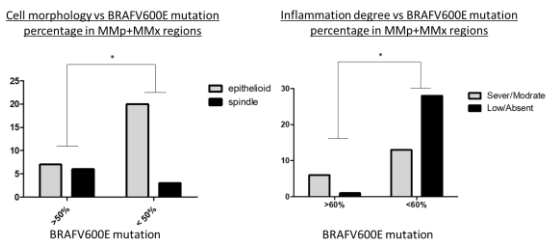

Figure 5) Fisher exact test showed statistical significance (p-value=0.0459) between spindle and epithelioid regions when the percentage of BRAFV600E mutation cut-off was established at 50%.

Figure 6) Fisher exact test showed statistical significance (p-value=0.0114) between severe/moderate and low/absent inflammation regions when the percentage of BRAFV600E mutation cut-off was established at 60%.

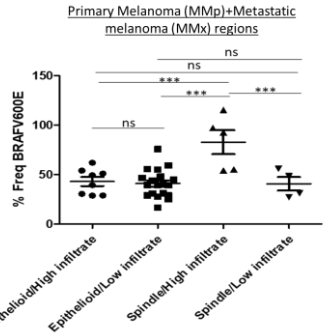

Figure 7) When cell morphology and inflammation were paired, regions with high/moderate inflammatory infiltrate had significant higher percentage of BRAFV600E mutation in the fusiform regions than epithelioid regions. Significant difference between high and low infiltrate was only observed in fusiform regions. Significant difference between epitheliod and fusiform was observed in high infiltrate. (**p<0,001).

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