Aggressiveness of melanoma subtypes selected by reflectance confocal microscopy

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Introduction

Malignant melanoma (MM) can present with different clinical, histopathological and biological aspects, suggesting an heterogeneous family of diseases rather than a unique entity (Clark et al., 1969, Zalaudek et al., 2008). In fact, MM is characterized by several cell populations that show diverse genotypic and phenotypic features, signalling pathway, biological behaviour and response to therapy. Traditionally, MM have been classified on the basis of growth pattern and biologic behaviour (Clark et al., 1969), but this classification is insufficient to provide enough information about causative factors or to select patients for specific treatment.

Reflectance confocal microscopy (RCM) allows to analyse architectural and cytological features at nearly histological resolution, and to visualize pigmented cell morphology better than conventional histopathology. This innovative approach offers an in vivo and in real time window into the tumor tissue when the lesion is still on patient's skin (Pupelli et al., 2013).

Pellacani and co-workers, by correlating RCM morphology with tumor and patient characteristics, have proposed the presence of four distinct melanoma subtypes (Pellacani et al., 2014). We hypothesize that RCM melanoma subtypes could present a different biological behaviour associated with a diverse degree of aggressiveness, which could also account for different response to therapy.

Objective

The aim was to study the biological behaviour of malignant melanoma RCM-subtypes

Materials and Methods

- 1) *Reflectance confocal microscopy*: analysis of 85 patients with malignant melanoma
- 2) *Immunohistochemistry*
- 3) *NanoString technology*: RNA extracted from paraffin-embedded melanoma tissues were analysed for the expression of 770 genes

Results

Representative Reflectance Confocal Microscopy features

85 melanoma were retrospectively evaluated by RCM. Lesions were classified in according with their morphology in: (A) **DC**, for the presence of a dendritic cells population (red arrow), (B) **RC**, which present roundish large melanocytes with dark nuclei in the epidermidis (red arrow), (C) **CT** for the presence of a mixed dendritic and round cells at epidermal layer, with dark nuclei (green and red arrow) and (D) **DN**, which presents cerebriform nesting located at dermal level (red arrow).

Clinical, Dermoscopic and Histopathological features

Association between RCM groups and patients/tumors characteristics

	DC	RC	CT	DN	Total
Age	66 ± 13.12	56.08 ± 17.17	65.19 ± 19.7	59.9 ± 14.09	61.79
Sex					
Female	8/25 32%	13/25 52%	9/25 36%	4/10 40%	40%
Male	17/25 68%	12/25 48%	16/25 64%	6/10 60%	60%
Tumor Type					
Radial growth	21/25 84%	14/25 56%	4/25 16%	1/10 10%	41.5%
Vertical growth	4/25 16%	11/25 44%	21/25 84%	9/10 90%	55.5%
Location					
Trunk	14/25 56%	14/25 56%	10/25 40%	4/10 40%	48%
Extremity	4/25 16%	11/25 44%	10/25 40%	4/10 40%	35%
Facial	7/25 28%	0/25 0%	5/25 20%	2/10 20%	17%
Breslow index					
<5	25/25 100%	25/25 100%	20/25 80%	8/10 80%	90.0%
5-10	0/25 0%	0/25 0%	5/25 20%	1/10 10%	7.5%
>10	0/25 0%	0/25 0%	0/20 0%	1/10 10%	2.5%
Thickness mean	0.72	0.66	2.53	5.65	2.39
Clark Level					
I	13/25 52%	0/25 0%	0/25 0%	0/10 0%	13.0%
II	7/25 28%	15/25 60%	3/25 12%	1/10 10%	27.5%
III	3/25 12%	9/25 36%	7/25 28%	3/10 30%	26.5%
IV	2/25 8%	1/25 4%	13/25 52%	6/10 60%	31.0%
V	0/25 0%	0/17 0%	2/25 8%	0/10 0%	2.0%
Mitotic Number					
0-1	23/25 92%	23/25 92%	10/25 40%	4/10 40%	66%
2-5	1/25 4%	2/25 8%	9/25 36%	4/10 40%	22%
>5	1/25 4%	0/25 0%	6/25 24%	2/10 20%	12%
TOTAL	25	25	25	10	85

Expression of melanoma, prognostic markers and BRAF mutation

Expression of aggressiveness and stemness markers

Expression was scored 0 to 4 and the average positive cell was calculated for each individual melanoma as follow:

- 1) 0 score=0%
 - 2) 1 score=1-25%
 - 3) 2 score=26-50%
 - 4) 3 score=51-75%
 - 5) 4 score=76-100%
- staining positive melanoma cells

Heatmaps of differentially expressed genes in DC, RC, CT and DN lesions

Genes were clustered based on DAVID GO functional annotation tool analysis (<https://david.ncicrf.gov/>). Expression values are ranked according to their z-score after normalization. All heatmaps were generated with the R programming environment using Wards method with Euclidean distance (<http://www.r-project.org/>).

Conclusions

Taken together, these results show a progressive aggressiveness from DC to CT MM subtypes, while DN seems to have a unique behaviour.