CONTRIBUTION OF UNDIFFERENTIATED CELLS TO METASTASIS IN NEUROBLASTOMA

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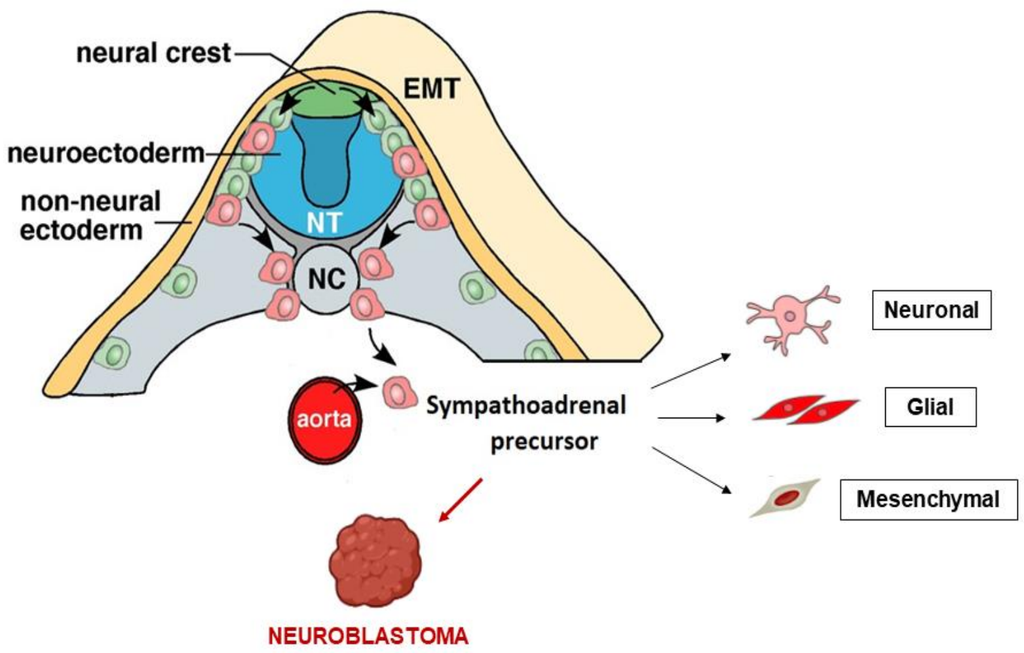
XIX CONGRESO de la SEBC
BOADILLA DEL MONTE (Madrid)
26, 27, 28 y 29 de octubre de 2021

INTRODUCTION

Neuroblastoma (NB) is the most common extracranial solid tumor in childhood. This disease arises from the neural crest components of the sympathetic nervous system and presents a high clinical heterogeneity ranging from spontaneous regression to aggressive metastasis [1]. Cellular heterogeneity has also been observed, including two types of tumor cells with divergent gene expression profiles and phenotypes: undifferentiated mesenchymal cells (MES) and committed adrenergic cells (ADRN) [2].

Aggressive NB tumors contain a population of undifferentiated cells that exhibit stem cells properties: the cancer stem cells (CSC). Conceptually, CSCs that evade intensive multimodal therapy dictate tumor progression, relapse/recurrence, metastasis formation, and poor clinical outcomes [3]. The formation of these aggressive metastases takes place thanks to a multistage process in which cell migration is essential.

Migratory cancer cells undergo dramatic cellular and molecular changes by remodeling their cell-cell and cell-matrix adhesion, as well as their cytoskeleton. Changes in cell shape are translated and are indicative of biochemical signals that can modulate cell phenotype and biological properties. These changes in the shape are indicative of the behavior and evolution of cells. Indeed, cell morphology has been shown to regulate biological processes, such as proliferation, differentiation, and the fate of stem cells [4]. A greater understanding of the molecular pathways that determine the mode of motility used by cancer cells and how the change between different architectures of the cytoskeleton is regulated will be very beneficial in understanding why and how cancer cells leave primary tumors to invade different tissues. [5]



1. MORPHOLOGICAL ANALYSIS

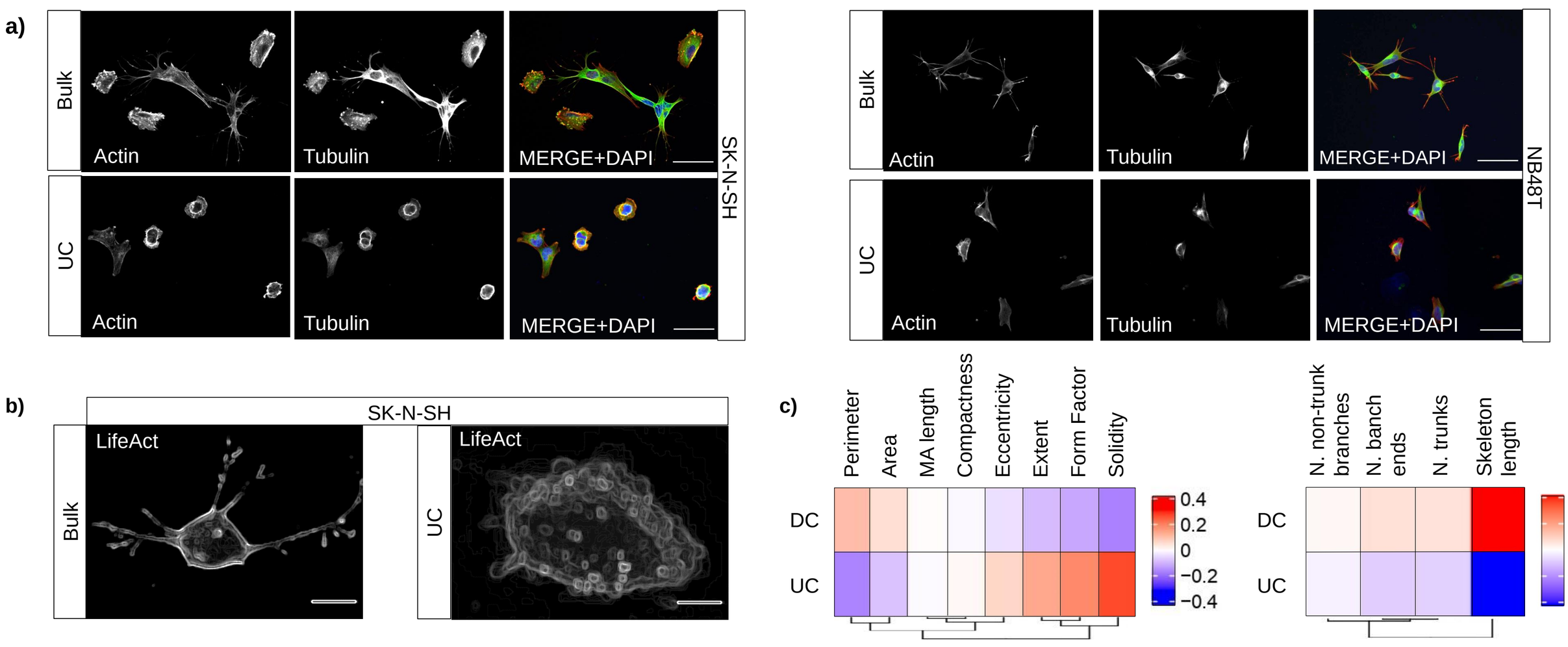


Figure 1. Morphological analysis of the SK-N-SH and NB48T cell lines. a) Representative confocal fluorescence maximum projections of tumoral cells in 2D or **b)** embedded in 3D matrigel. The bulk of tumoral cells show more neuronal characteristics, while undifferentiated cells show more mesenchymal characteristics. Scale bar: 50µm (a) and 10µm (b). **c)** Heatmaps of morphological characteristics of undifferentiated cells (UC) and differentiated cells (DC): perimeter, area, major axis (MA) length, compactness, eccentricity, extent, form factor, solidity, number (N.) of non-trunk branches, number of branch ends, number of trunks, and total skeleton length. It is observed that undifferentiated cells, in comparison to the rest of tumor cells, are smaller with less perimeter, higher solidity, form factor, extent and eccentricity, and have fewer and shorter neurites.

2. MIGRATION AND CHEMOTAXIS ANALYSIS

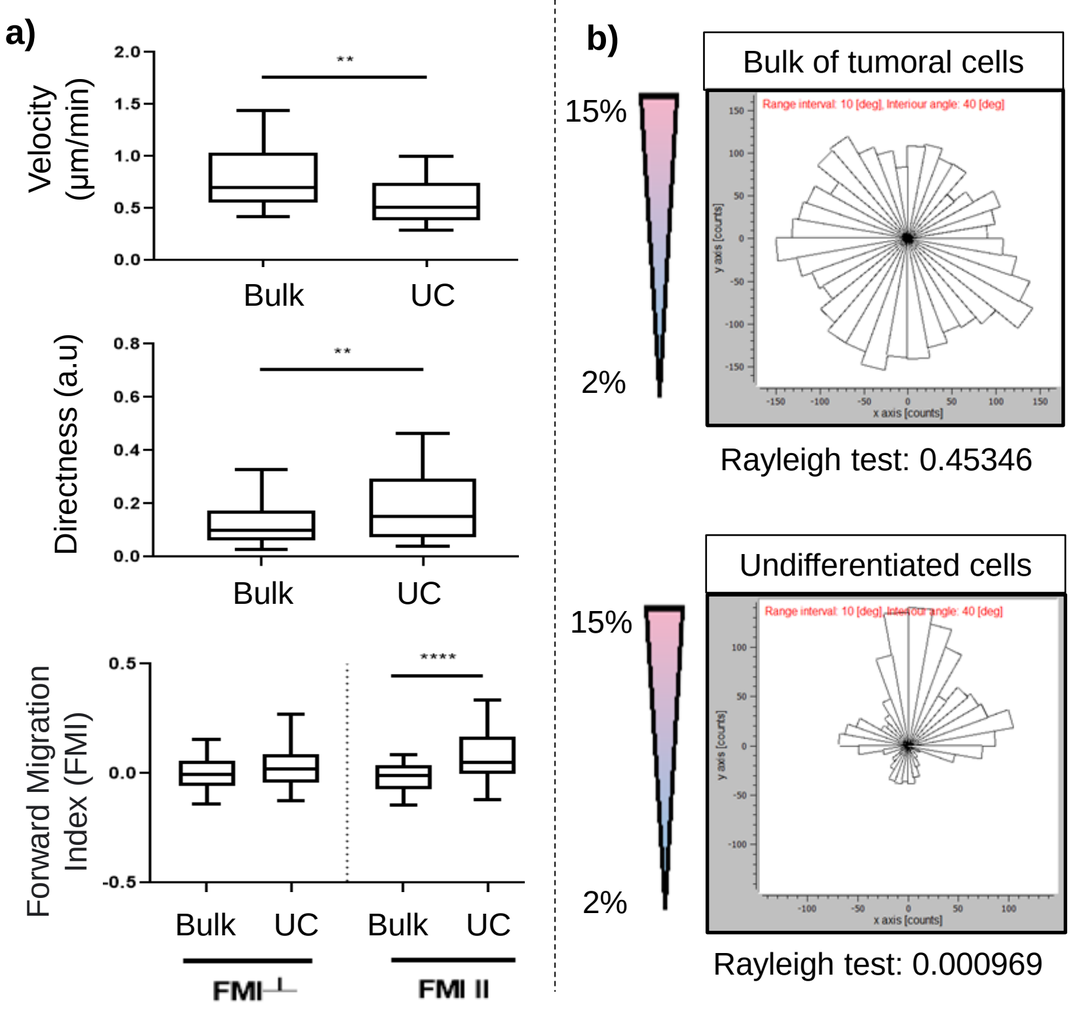


Figure 2. Migration analysis of SK-N-SH cells. a) Quantification of the speed, directness and forward migration index of the bulk of tumoral cells and undifferentiated cells (UC) in the presence of a chemotactic gradient. Undifferentiated cells move slower, with high directness and FMI II (in a direction parallel to the gradient). **b)** Graph of the migration trajectories in the x/y axis. Undifferentiated cells show a directed, chemotactic migration.

3. MEMBRANE DYNAMICS ANALYSIS

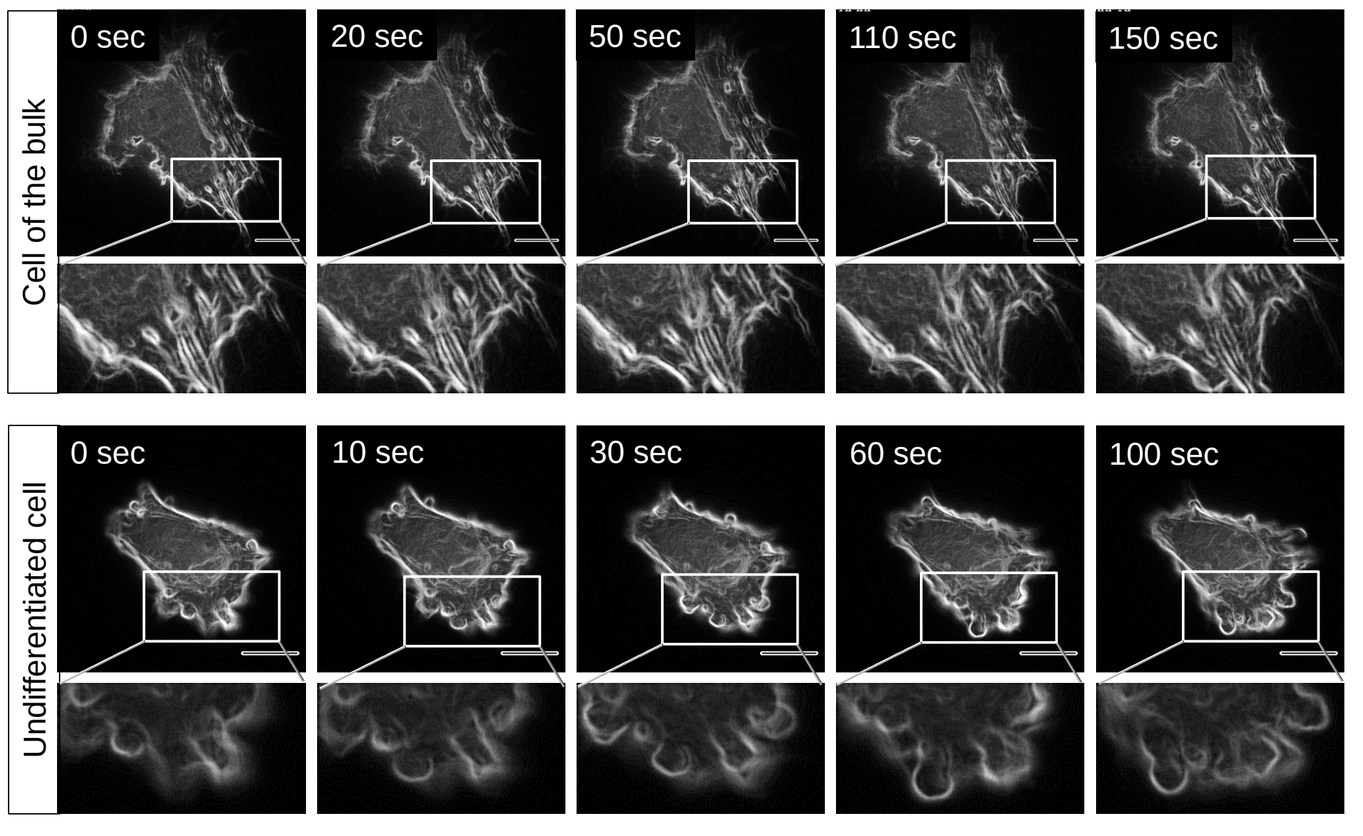


Figure 3. Analysis of the membrane dynamics of SK-N-SH cells. a) Immunofluorescence images of a cell of the bulk of tumoral cells and an undifferentiated cell, both marked with Lifeact. Undifferentiated cell displaying membrane blebs, while the rest of the tumoral cells display predominantly filopodia. Scale bar: 10µm.

CONCLUSIONS

- The most undifferentiated cells of the tumor show great cellular plasticity.
-The most undifferentiated cells of the tumor have a particular morphology and cytoskeletal structure.
- The most undifferentiated cells of the tumor are characterized by having a greater migratory and chemotactic capacity than the bulk of tumoral cells.
- The more undifferentiated cells of neuroblastoma may have important metastatic characteristics.

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