

Innovative experimental approach for the morphological characterization of cancer stem cells spheroids

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It's widely accepted the involvement of EMT in the onset of cancer metastasis given its ability to enable both cancer cells dissemination and self-renewal. Many researches have showed that the generation of circulating tumor cells (CTCs) with stem cells-like properties is responsible for metastasis successful; despite the advances in CTCs detection systems, the molecular characterization appears tough since their existence in a very small amount. For this purpose, increasing numbers of studies have developed multiple methodological tools for the culture of cancer cells exhibiting stem cells-like properties, including 3D-spheroids propagation [1]. Our group have previously showed that ferritin heavy chain (FHC) exerts a negative role on both ovarian cancer stem cells expansion and EMT, via the application of the in vitro 3D spheroid assay [2]. Here, we applied an innovative experimental approach for the characterization of cancer stem cell spheroids. FHC-silenced (shFHC) and control shScr SKOV3-cells were cultured in ultra-low attachment plates and maintained with RPMI supplemented with 10% FBS. The first generation of spheroids derived from a 10-day cultures of FHC-silenced and control shScr SKOV3-cells were characterized for their number, size and morphology. The expression of proliferative markers, extracellular matrix components and stem cells markers was evaluated in both adherent and spheroids cells using the cell block technique. First, shFHC cells showed a significant greater number and larger 3D spheroids than control shScr SKOV3-cells in 10-day cultures. Proliferative activity as determined by Ki-67 immunoreactivity showed an even distribution in adherent cells. Otherwise, in FHC-silenced spheroids, proliferation was predominant in the peripheral areas. The spheroid cell cultures also exhibited a distinct network of CD44 and CD56. We suggest our experimental approach as a useful tool for testing the role of FHC in the acquisition of stem cells-like properties.

References

- [1] Ishiguro T et al. (2017) Tumor-derived spheroids: Relevance to cancer stem cells and clinical applications. *Cancer Sci* 108(3):283-289.
- [2] Lobello N et al. (2016) Ferritin heavy chain is a negative regulator of ovarian cancer stem cell expansion and epithelial to mesenchymal transition. *Oncotarget* 7(38):62019-62033.

Key words

Cancer stem cells spheroids, cell block technique, ferritin.