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Introduction

Glioblastoma Multiforme (GBM) is the most common tumor that emerges from the central nervous system. It has an incidence of 2–3 cases per 100,000 people in Europe and North America. GBM de es modern treatments such as surgery, radiotherapy [1] and chemotherapy [2] and consequently, median survival ranges from just 9 to 12 months [3]; and 5-year mortality rates are as high as 95%. GBM primary tumors can develop a er a short clinical history and without any evidence of a precursor lesion [4]. In contrast, secondary tumors develop from the progression of low-grade astrocytomas.

e cancer stem cells (CSC) hypothesis [5] is, currently, the most widely accepted theory regarding tumor formation and self-renewal ability. It states that there are di erent tumorigenic phenotypes inside a tumor mass. One of these cell phenotypes is capable of generating new tumors if transplanted to a host and it is able to self-generate and regenerate the rest of the tumor cells [6,7]. is cell type is called CSC in accordance with the similarities found with stem cells. e fact that CSC are able to withstand a large number of drugs and treatments, and have the ability to regenerate the tumor mass a er treatment, makes them a highly attractive target for new therapies and anti-tumorigenic drugs [8]. e need to nd pharmacological compounds capable of eliminating CSC makes the search for methods of recognition and isolation of these cells for experimentation a matter of great urgency.

qPCR detection system (Bio-Rad), with 2.5 l of starting cDNA. For quanti cation, an ef ciency corrected quanti cation model was applied. e derivative ratio values describe the relative expression change of the target gene relative to the GAPDH reference gene expression:

neurosphere groups had higher levels of expression of Nestin, Musashi1, CD133 and SOX2 genes [13,14]. On the other hand, the CD133- and the monolayer groups presented almost the same expression levels of Nestin and CD133, as expected [13,14], but higher levels of the stem gene markers SOX2 and Musashi1. ese results may be explained by three facts: rst, CD133- cells may have changed to CD133+ during the passages of the culture a er sorting; second, the existence of partially di erentiated CSC in the CD133- group, which do not express CD133 but remain pluripotent and express stem gene markers [31]; and nally, it is also plausible that the sorting of CD133+ cells was not speci c enough, leaving some of them in the negative fraction. e RT-qPCR results of the expression of GLI1 showed ambiguous di erences among the groups. e neurosphere and the CD133+ groups had the higher levels of expression, 2 fold and 1.6 fold, when compared to the monolayer group. But the di erences are not as evident as in the other RT-qPCR experiments.

With regard to the ow cytometry experiment, the so agar colony formation and especially the RT-qPCR assays make us doubt the validity of the protocol set for the FACS technique. One question that might be taken into account is whether it is possible that a CD133- cell fraction can grow in SFM when previous studies have shown that CD133- cells have reduced ability to form neurospheres [2,25,30]. e sorted CD133- cells used in this analysis could grow in SFM, which lead us to the conclusion that, maybe, a CD133- cell does not necessarily have to be excluded from being a CSC. In the same way, a CD133+ group of cells does not necessarily mean a group of CSCs. Perhaps a higher probability of nding one CSC in a CD133+ is more convincing than just calling any CD133+ cell a CSC.

Acknowledgements

:H WKDQN /DXUD 6WRNHV IRU HGLWLQJ RI WKH PDQXVFULSW DQG WKH GLUHFWRU RI &,)$ IRU VKDULQJ ODERUDWRULHV 7KLV UHVHDUFK ZDV VXSSRUWHG LQ SDUW E\ JUDQWV IURP WKH 'HSDUWPHQWR GH 6DOXG GHO *RELHUQR GH 1DYDUUD &DMD 1DYDUUD SURMHFW)XQGDFLyQ 8QLYHUVLWDULD GH 1DYDUUD 3DPSORQD DQG)RQGR GH ,QYHVWLJDFLyQ 6DQLWDULD 3, WR -6& DQG 3, WR -$5 0DGULG

References