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A novel method for the establishment of primary cultures of cancer stem cells

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Li-Fraumeni syndrome (LFS), an inherited cancer predisposition syndrome, is associated with germline mutations in *TP53*. It is characterized by high risk of multiple, early cancers. In Brazil, a variant form of LFS is exceedingly frequent due to a widespread founder *TP53* mutation, p.R337H, detected in about 0.3% of the general population in Southern Brazil. This mutation occurs in p53 oligomerization domain and its effect on p53 oligomerization is supposed to be dependent upon pH conditions. Recent studies indicate that p53 plays a critical role in regulating differentiation and asymmetric divisions of stem cells. The main objective of this study is to isolate and to characterize Cancer Stem Cells (CSC) in patients with germline mutations in TP53 gene with Li-Fraumeni syndrome and Li-Fraumeni-*like* Syndrome. We have isolated and characterized CSC from tumors of p.R337H mutation carriers. After informed consent, surgical resection fragments were dissociated and brought in culture. Adherent cells and spheroids were derived from different tumor types. Spheroids and stromal cells derived from a breast cancer (BC) were further analyzed by immunofluorescence and flow cytometry to demonstrate positive immunolabeling for $CD44^+$, $CD24^-$, Oct4, Ki67 and Sox2 antibodies. Time-lapse videomicroscopy showed rapid growth, frequent asymmetric division and absence of senescent phenotypes for least 17 passages. Moreover, we showed by Colony Forming Units assay (CFU) that stromal cells are very clonogenic, once 10^3 cells initially plated were able to form 442 new colonies after 15 days in culture. It was also verified the differentiation potential of stromal cells for the following lineages: adipogenic, osteogenic and muscle-like cells. The adherent stromal cells as well as oncoesferas are able to self-renew and to proliferate in vitro for long periods without losing its main features. These properties are similar to stem cell possessing the p53 inactive. However, we observed that treatment of adherent cells with doxorubicin (DNA damaging agent) causes accumulation of p53 and causing induction of cell cycle arrest with low doses of this drug, and cell death / apoptosis in higher doses. Our findings using CSC-p.R337H can provide a very useful model for better understanding the role of specific mutation, as well as the TP53 tumor biology.

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