The use of Bottlenose Dolphin umbilical cord mesenchymal stem cells in regenerative medicine and diagnostic tests

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Introduction: Mesenchymal stem cells (MSCs) have become a promising tool due to their peculiarity of being undifferentiated and pluripotent cells that can differentiate into many specialized cells. In marine mammals, studies on stem cells are sporadic, and there are little references available. To increase the knowledge of the stem cells in the cetaceans it is important to have the availability of established cell lines to use for toxicology and diagnostic assays. Here we describe the isolation, differentiation and immortalization of a cell line derived from the umbilical cord (UC) of a captive female bottlenose dolphin (Tursiops truncatus) collected during labour.

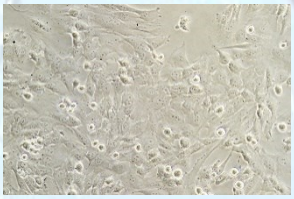

Fig.1: The DOC-hTERT cell line is derived from transfection of the primary DOC culture, obtained from dolphin umbilical cord (Tursiops truncatus) with a vector plasmid (pBABE) containing the Hygromycin resistance gene and the hTERT gene sequence, codifying for a ribonucleoprotein polymerase (Telomerase) telomere TTAGGG.
Uncoloured 20X MO cells of which retains the length of telomere ends by adding repeat .

Conclusions: The DOC-MSC provides a novelty *in vitro* model for cell and gene therapy, cloning and biotechnological applications. DOC-MSC can constitute the basis for biological treatments in wound healing and tissue regeneration in dolphins. In addition, due to the growing interest in viral diseases of aquatic mammals, these cells could represent the ideal substrate for in vitro virus isolation. Indeed, DOC-MSC could also be used as a fundamental tool for toxicology assays, as an alternative to animal models, to identify the cytotoxicity levels caused by chronic exposure to environmental pollutants (organochlorines and heavy metals), providing valuable information regarding conservation and protection of marine mammals.

Results and discussion: In the laboratory, UC was submitted to enzymatic collagenase digestion (IA·Sigma-Aldrich) and was resuspended in growth medium (DMEM + 10% FBS; 100 U/ml penicillin and 100 µg/ml streptomycin, Sigma-Aldrich) at 37 °C with 5% CO_2 atmosphere. Cells isolated from the dolphin's umbilical cord (DOC) can proliferate and generate homogeneous colonies as well as prove to meet all the criteria for stem cells: cells are plastic adherent and they have a spindle-shaped morphology. Evaluation of osteogenic, chondrogenic and adipogenic differentiation, after addition of specific differentiation media, was performed by cytochemical staining to confirm presence of calcium production, intra- and extracellular glycosaminoglycan residues and intracellular accumulation of lipid-rich vacuoles. DOC-derived cells were transfected with a plasmid encoding the hTERT, adding the hygromicin-resistance gene (Addgene #1773). All hygromicin-resistant clones were isolated and expanded, and all of them proliferated at a faster rate than non-transfected DOC cells, which exhibited signs of senescence after 32 passages.

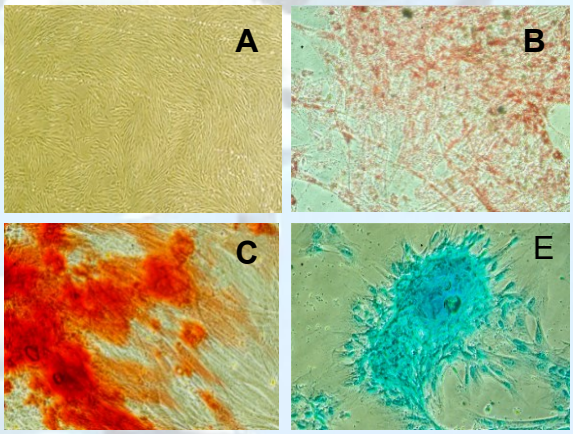

Fig 2: DOC-MSC grown without the differentiation medium (A). DOC-MSC in specific differentiation medium: Oil Red-O (B), Alizarin Red S (C), Alcian blue (D) stained
Optical microscopy images (x20)

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