INNOVATIVE APPROACHES TO ESTABLISH AND CHARACTERIZE PRIMARY CULTURES: AN EX VIVO 3D SYSTEM AND THE ZEBRAFISH MODEL

Chiara Liverani[1,#], Federico La Manna[1,2,#], Arwin Groenewoud[3], Laura Mercatali[1], Gabri Van Der Pluijm[2], Federica Pieri[4], Davide Cavaliere[5], Alessandro De Vita[1], Chiara Spadazzi[1], Giacomo Miserocchi[1], Alberto Bongiovanni[1], Federica Recine[1], Nada Riva[1], Marina Faedi[1], Sebastiano Calpona[1], Davide Bruschi[1], Dino Amadori[1], Ennio Tasciotti[6], Marcantognini Giulia[1], Fausti Valentina[1], Martina Ghetti[1], Ewa Snaar-Jagalska[3,‡] and Toni Ibrahim[1,‡,*]

[1]Osteoncology and Rare Tumors Center, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, via P. Maroncelli 40, Meldola, Italy. [2]Leiden University Medical Center, Department of Urology, J-3-100, Albinusdreef 2, Leiden, The Netherlands. [3]Department of Molecular Cell Biology, Institute of Biology, Leiden University, Sylviusweg 72, Leiden, The Netherlands. [4] Pathology Unit, Morgagni-Pierantoni Hospital, 47121 Forlì, Italy. [5]Unit of Surgery and Advanced Oncologic Therapies, Morgagni-Pierantoni Hospital, Forlì, Italy. [6]Department of Regenerative Medicine, Houston Methodist Research Institute, 6670 Bertner Avenue, Houston, TX, USA.

*Author for correspondence (toni.ibrahim@irst.emr.it).

[#] These authors contributed equally to this work.

[‡] These authors contributed equally to this work.

Abstract

Patient-derived specimens are an invaluable resource to investigate the heterogeneous biology and behavior of cancer cells, in particular in rare tumors as Soft Tissue Sarcomas, for which several subtypes exist, not all covered by the matched cell lines. However, *in vivo* studies on primary cultures are often limited by the small amount of material available, while conventional *in vitro* models might alter the features that characterize cancer cells. Here we used a 3D scaffold-based system and the Zebrafish model to isolate and study a primary dedifferentiated liposarcoma culture. Cells were characterized *in vitro* for morphological features, sensitivity to drugs and biomarker expression, and *in vivo* for their engraftment and invasiveness abilities. The 3D culture showed a higher enrichment in cancer cells than the standard monolayer culture and a better preservation of liposarcoma-associated markers (Fig 1a). 3D-enriched cells proved sensitive to drugs with survival

percentages not significantly different between epirubicin plus ifosfamide and trabectedin. Liposarcoma cells injected in the Zebrafish were successful engrafted in the heart cavity and in later stages were scattered throughout the implantation area showing local migratory and invasive abilities (Fig 1b). Our work provides proof of concept of the ability of 3D cultures to maintain the original phenotype of ex vivo cells, and highlights the potential of the Zebrafish to provide a versatile model for *in vivo* studies with limited biological material. Such models could be used in translational research studies for biomolecular analyses, drug screenings and tumor aggressiveness assays.

Figure 1