Innovative TEM techiniques for ultrastructural characterization of human colon cancer stem cell-derived spheroids and xenograft in a mouse model

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Colorectal cancer is the third most common malignancy diagnosed worldwide and one of the major cause of cancer death in developed countries, with broad diffusion and increasing incidence. Despite emerging therapies and advances reached in the last years more than 30% of patients relapse and develop metastasis for acquired resistance. Cancer stem cells represent the population of the tumor responsible for recurrence of the disease, metastatic spread and are resistant to currently available therapies. Human colorectal cancer biopsies, obtained during surgical procedures after patient informed consent, were cultured in a selective medium to enrich a line of colon cancer stem cells (CCSCs) multicellular spheroids (CCSC-L1). Some multicellular spheroids were fixed and stored at 4°C in glutaraldehyde 2,5% for electron microscopy study and other were subcutaneously injected in in 5 immunocompromised NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (NSG) mice. Mice were sacrificed after 3 weeks, when cancer stem cell-derived xenograft reached dimensions of about 100mm3 . Samples were fixed immediately post recovery in glutaraldehyde 2,5% in pbs and were then prepared for transmission electron microscopy with a special protocol that enhances membranes, osmium 2% followed by 1% tannic acid. Semithin sections were observed at VP SEM after being observed at LM in order to obtain high power view of a large observation field. CCSCs spheroids appear as multicellular structures with cells in various stages of differentiation but lacking of goblet cells or enteroendocrine cells. CCSCs-derived xenograft showed the same morphology of colon cancer, it appeared well vascularized and innervated with a connective tissue envelopment rich in fibroblast. The xenograft showed mainly differentiated enterocytes but also cells in various stages of differentiation and cells that are under epithelial mesenchymal transition. No goblet cell or enteroendocrine cells were observed. This is the first ultrastructural study of CCSCs multicellular spheroid and their xenograft from the cellular line CCSC-L1. Indifferentiated cancer cells and fully differentiated enterocytes were observed in both spheroids and xenograft, as well as goblet cells and enteroendocrine cells were absent in both samples. Epithelial mesenchymal transition instead was observed only in the xenograft, which is enveloped by connective tissue, innervated and vascularized, this underline the importance of a supportive in vivo microenvironment, whose influence is absent in multicellular spheroids.

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