

Ultrastructural characterization of human colon cancer stem cell-derived spheroids and xenograft in a mouse model

Michela Relucenti¹, Paola Contavalli², Selenia Miglietta¹, Ezio Battaglione¹, Vito d'Andrea³, Federica Francescangeli², Ann Zeuner², Giuseppe Familiari¹

¹ La Sapienza Università di Roma, Dip. SAIMLAL, Roma, Italia (1) –

² Istituto Superiore di sSnità, dipartimento di Oncologia e Medicina molecolare, Roma, Italia (2)

³ La Sapienza Università di Roma, dip Scienze Chirurgiche, Roma, Italia

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 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 conventional scanning and transmission electron microscopy. CCSCs spheroids were observed by means of scanning and transmission electron microscopy, they were formed by 8-10 cells. In these multicellular structures colon cancer stem cell, mitotic figures and differentiated enterocytes were observed. No goblet cells or enteroendocrine cells were found. 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 stem cells 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. Cancer stem 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.

Con il contributo del Ministero degli Affari Esteri e della Cooperazione Internazionale, Direzione Generale per la Promozione del Sistema Paese

Key words

Colon cancer, stem cells, electron microscopy, epithelial mesenchymal transition, metastasis.