Histology and Histopathology
From Cell Biology to Tissue Engineering

P01 - Ultrastructural study of rabbit VX2 liver tumor: metastasis and necrosis

Junquera C.[1,2], Aramayona J.C.[3], Borja P.[4], Güemes A.[2,5], Baselga M.[2]; Arribas D.[2,5], Briz P.[4], Lucía O.[4], Burdio J.M.[4]; Monleón E.[1,2]

[1] Department of Human Anatomy and Histology, University of Zaragoza, Zaragoza, Spain.
[2] Institute for Health Research Aragon (IIS Aragón), 50009, Zaragoza, Spain.
[3] Department. of Pharmacology and Physiology, University of Zaragoza, Zaragoza, Spain
[4] Department of Electronics Engineering and Communications, University of Zaragoza, Zaragoza, Spain
[5] Department of General Surgery, Hospital Clínico Universitario Lozano Blesa, Zaragoza, Spain.

Introduction: Since its introduction over 70 years ago, the rabbit VX2 carcinoma has been extensively used as a model of liver tumors to assess various treatment modalities that require animals larger than rodents. The VX2 tumor is a squamous cell carcinoma derived from *Shope papillomavirus* infection in rabbits, characterized by rapid growth. Its implantation techniques or growth characteristics have been accurately described, but information about its biology is scattered, to date, no ultrastructural study of the model has been carried out. The objective of this work is perform an analysis of the biological processes that lead to metastatic development and tumor necrosis.

Methods: To follow the development of the tumor we have carried out a tumor implant in 8 adult New Zealand white rabbits (weight: 2.5–3.0 kg, male) which were sacrificed in pairs at 4, 8, 11 and 17 days after implantation. Fresh tumor tissues were obtained from VX2 tumor-bearing rabbits that were subcultured in our laboratory. Briefly, fresh tumors were cut into small pieces of 1 mm3 under aseptic conditions, then a piece of tumor was implanted orthotopically into the middle liver lobe adjacent to the gall. The tumors were dissected within the established time and the biopsies were processed to perform histological and electron microscopy techniques according to conventional protocols. All the animals were used under approved animal care and using committee protocols.

Results: After 8 days of growth tumors are well delineated and composed of lobules of large undifferentiated cells with a high nucleo- cytoplasmic ratio. Their nuclei are large, round, and with prominent nucleolus that, due to its high activity in the synthesis of ribosomal particles, sometimes extends to the nuclear envelope. In the cytoplasm of tumor cells, few granular endoplasmic reticulum saccules, few mitochondria and a large number of free polyribosomes are observed

(Fig.1). The cells are closely linked to each other by desmosomes characterized by their large bundles of tonofilaments. The different lobules of tumor cells appear covered by a narrow fibrous layer formed mainly by fibroblasts. After 11 days of tumor growth, we can observe the ultrastructural characteristics of the epithelial-mesenchymal transition (EMT) process, associated with the first steps of the metastasis. Tumor cells must break loose and acquire a mesenchymal phenotype in order to migrate. The rupture of the desmosomes is visualized by the separation of the cell membranes, and the appearance in their cytoplasm of the detached tonofilaments. The presence of autophagic vacuoles is also notable. From the periphery of the tumor, the cells are released, gradually acquiring a mesenchymal phenotype that allows them to move. Some of them present primary cilium. Intermediate phenotypes are also observed. From 14 days the largest tumor sheets or lobules are generally centered by necrotic areas containing necrotic and apoptotic-cell debris, and few inflammatory cells, mostly macrophages and lymphocytes. A cytoplasmic vacuolization begins to be observed that corresponds to a mitochondrial alteration, due to lack of oxygen, in which the rupture of the cristae and even the mitochondrial membranes occur.

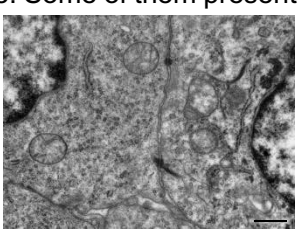

Fig.1. Ultrastructural characteristics of tumor

Discussion & Conclusions: Our findings establish two temporal parameters, metastasis and necrosis, that affect tumor survival. These data will serve to guide the design of future preclinical research using this model.

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