Histology and Histopathology

From Cell Biology to Tissue Engineering

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Index

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Ultrastructural study of pig liver regeneration following ablation with irreversible electroporation

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Irreversible electroporation (IRE) provides an increase in cell membrane permeability following exposure to high-voltage that leads to cell death. IRE has emerged as a promising tool for treatment of multiple diseases including hepatic cancer. However, tissue regeneration mechanisms following IRE ablation have not been investigated yet. In a previous work, we analyzed pig liver tissue response following IRE ablation. Now we try to determine the cellular events that take place in the process of liver regeneration at ultrastructural level. Our findings suggest IRE ablation as a good model to study liver regeneration.

In this study, 4 pigs (40 kg) were treated using parallel-plate electrodes separated by the width of the liver (i.e. 1-1.5 cm) and applying 100 pulses of 100 µs, with a frequency of 0.5 Hz to avoid thermal damage, and 2000 V/cm electric field intensity. Post-operative biopsies were performed at 7 and 14 days after the stimulation. Samples were routinely processed for transmission electron microscopy visualization.

Seven days after electroporation, numerous activated hepatic stellate cells were positioned in the same direction within a collagen-rich extracellular matrix. These cells show long cytoplasmic projections, voluminous nuclei with peripheral heterochromatin and displaced nucleoli near the nuclear envelope. Cytoplasmic projections contact with neighbor cells originating a complex net. Abundant rough endoplasmic reticulum, polyribosomes and cortical actin filaments were found. Some of these cells show activated centrioles migrating from their usual perinuclear situation to cell membrane, where they form a primary cilium. Next to these cells, we found another, less frequent, cell population composed by rounded or oval cells with more electrondense cytoplasm and abundant polyribosomes. These cells show fine projections that contact with stellate cells.

Both types of cells stayed around of new-formed biliary ducts composed by cells with voluminous nuclei, numerous polyribosomes and small reticulum cisternae; cellular junctions begin to be established between these cells. Formation of small blood vessels is also observed. These new blood vessels are originated from cells with similar features that those forming biliary ducts. Hepatic lobules appeared later and hepatic cells showed some immature characteristics, like round scarce mitochondria, even fourteen days after IRE.

Hepatic stellate cells show primary cilia that may initiate cell differentiation through external signals. Hepatic regeneration following IRE ablation is sequential: biliary ducts and blood vessels are already formed at seven days while newborn hepatocytes appear later, around fourteen days after IRE.