S3-06

Application of Confocal Laser Scanning Microscope on the Cell Biological Study

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Rapid stretch injury (RSI) is an in vitro model that mimics many features of experimental traumatic brain injury in vivo. Gap junctions (GJ) contribute to vasodilatation and vasoconstriction and, perhaps, to cerebral vascular regulatory mechanisms such as autoregulation. Rat vascular smooth muscle cells (A7r5, ATCC) were seeded onto six-well FlexPlates (Flexcell International) that had a silastic bottom and provided the ability to deform and injure the cells. Mild, moderate and severe groups of RSI (5.5, 6.5, 7.5 mm deformation) were produced using a model 94A cell injury controller (Commonwealth Biotechnology). Cell injury was defined as the percent of Hoechst 33343 stained cells that stained with propidium iodide (PI). GJ communication was assayed using fluorescence recovery after photobleaching (FRAP). Cells were loaded with 5-carboxyfluorescein diacetate and intracellular fluorescence was measured using confocal fluorescence microscopy (Zeiss LSM510). FRAP was expressed as percent of baseline fluorescence. Intracellular Ca++ and ROS were imaged using the fluorescent dyes fluo-4 AM and H2DCFDA, respectively. RSI produced level dependent increases in intracellular Ca++ and ROS and PI staining and decreases in GJ communication. SOD or the Ca++ chelator, EGTA, improved GJ communication and reduced the percentage of PI stained cells. These results suggest that increases in intracellular Ca++ and ROS contribute to cell injury and impaired GJ communication after RSI in A7r5 cells in vitro.

S4-01

Application of immunohistochemistry for prognostication and molecular targeted therapy in patients with breast cancer and ovarian cancer

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Immunohistochemistry (IHC) is now routinely used as tests of expression of hormone receprors and HER2 (c-erbB-2) proto-oncogene for patients with breast cancers in order to identify eligibility to endocrine and trastuzumab therapies, respectively. Despite the development of various chemothrapeutic agents, mortality due to ovarian cancer (OC) has almost unchanged during recent ten years. More effective treatment options based on molecular pathways of tumor growth and metastasis are needed. HER2 also expresses in OCs, but its prognostic significance is controversial. Vascular endothelial growth factors (VEGFs) are frequently detected in OCs, and clinical trials using bevacizumab, an anti-VEGF antibody, are ongoing. Other possible molecular targets detectable with IHC for patients with OCs comprise platelet-derived growth factor receptors (PDGFRs), WT1 (Wilms' tumor 1), and actinin-4. PDGFRs, type III receptor tyrosine kinase that can be treated by imatinib mesylate, were frequently expressed and activated by autocrine manner in OCs. Ovarian serous adenocarcinomas were mostly immunopositive for WT1, and the intensity of WT1 immunoreaction was correlated with WT1 mRNA levels and patients' clinical outcome. Immunotherapy using vaccinization of WT1 protein is of interest. Actinin-4, an actin-bundling protein, that enhances cell motility, was also frequently expressed in OCs. High actinin-4 expression was correlated with serous histology and poorer prognosis. Development of target therapies against these molecules might contribute to decrease in mortality due to OCs.