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4TH MEETING OF YOUNG GENERATION OF VETERINARY ANATOMISTS

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4. SREČANJE MLADIH VETERINARSKIH ANATOMOV – YGVA

Pod pokrovitelstvom Evropske zveze veterinarskih anatomov EAVA
Ljubljana, Slovenija, 8. - 10. julij 2007
Sofinancirala Javna agencija za raziskovalno dejavnost Republike Slovenije

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Invited lectures – Vabljena predavanja

CONFOCAL MICROSCOPY: PRINCIPLES AND APPLICATIONS

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Background

The laser scanning confocal microscopy (LSCM) is an essential tool for many biomedical imaging applications at the level of the light microscopy. It enables multi dimensional imaging and optical sectioning of fluorescently labeled thick specimens and living cells. Argon ion and Helium/Neon laser beam of different wavelength is commonly used to excite fluorochrome present in the specimen rapidly, point by point in the x-y plane. The emitted fluorescent light of longer wavelength originating from the excited dye is then collected by the objective, directed through a small pinhole which rejects out of focus photons and reduces out of focus light. In this way thin and high quality optical section is generated with Z-resolution significantly improved compared to the conventional light microscopy. By changes in the focal plane serial of optical sections can be obtained from thick specimens and displayed as a digitalized images that allows subsequent 3-dimensional reconstruction (XYZ).

Material and methods

Liver cryo-sections and in vitro cultured rabbit embryos and in human embryonic kidney (HEK-293) cell line (European Collection of Animal Cell Cultures (Salisbury, UK)) were studied in a Leica multispectral LSCM (Leica TCS NT). The sequential detection of the microtubules labelled with indirect immunofluorescence, rhodamine-phalloidin-labelled actin filaments and the nuclei labelled with TO-PRO-3 iodide was achieved with the use of argon and helium-neon laser with excitation lines at 488, 543 and 633 nm. The data from the channels were collected sequentially using an oil immersion objective lens (Leica, Planapo 40◊N.A.=1.25) with fourfold averaging of single frame scan at a resolution of 1024 x 1024 pixels. When appropriate, Z-series were generated by collecting stacks of optical slices by using a step size around 1 μm in the Z-direction. Acquired images were analysed and presented by Leica Confocal Software (Lite Version, Leica Microsystem, and Heidelberg, Germany) and Adobe Photoshop 7.0 computer software, respectively. For the three-dimensional (3-D) reconstructions stacks were also exported and analyzed in Silicon Graphics by Imaris 3.0 (Bitplane).

Results and discussion

In our laboratory, we primarily use confocal microscopy to study cell's cytoskeleton distribution pattern in tissue samples as well as in in vitro cultured rabitt embryos (1) and in cell lines stably expressing individual members of membrane-bound G protein-coupled receptors (2). Examples of actin and microtubules cytoskeleton visualization are shown on Fig. 1. Technique of direct fluorescence using rhodamine-phalloidin demonstrated actin cytoskeleton in frozen liver tissue sections (Fig. 1a) and in paraformaldehyde fixed and with Triton X-100 permeabilised rabbit embryos (Fig. 1b). Indirect immunofluorescence using a mouse monoclonal anti-tubulin antibody was used to visualise microtubules distribution in HEK-293 cell line (Fig. 1c). The cell nuclei were stained with To-Pro-3 (Molecular Probes, Oregon, USA) for 30 min. Actin filaments are located under the cell membrane of hepatocytes (Fig. 1a) and blastomeres (Fig. 1b). In the cultured HEK-293 cells, the microtubule radiates through the cells from microtubule organization centers (Fig. 1c).

Over of the past decade, technological advancements in the LSCM have mainly encompassed improvements in the photon efficiency of the LSCM and continued development in digital imaging methods, laser technology and the availability of brighter and more photostable fluorescent probes. Such advances have made possible novel experimental approaches for multiple label fluorescence, live cell imaging and multidimensional microscopy. In conclusion, advantages of confocal microscopy which includes greater spatial

resolution over conventional fluorescence microscopy, optical sectioning of examined samples, 3-D images reconstruction and multi channels acquisition enabled widespred use of confocal microscopy in the cell biology imaging.

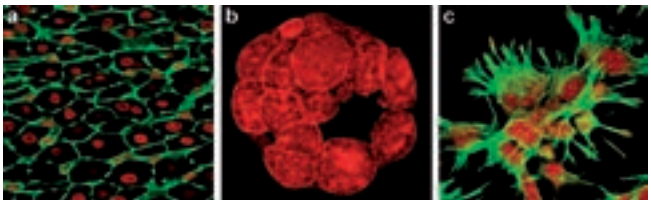

Figure 1: Confocal images of actin and microtubules cytoskeleton organisation. (a) confocal image of liver tissue section shows cortical actin organisation of rat hepatocytes (green signal) (b) actin cytoskeleton distribution in blastomeres of three days old rabbit embryo shown in red (c) cultured HEK-293 cells showing normal distribution of microtubule filaments (green signal). TO-PRO-3 iodide stained nuclei (a, c) are shown in red.

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MITOCHONDRIAL TRIGGERING OF CELL DEATH AND CONFOCAL MICROSCOPY

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The laser scanning confocal microscope detects images of multiple labelled fluorescent samples. One can follow intracellular distribution of the protein under investigation by tracing the location of fluorescently labelled protein or fluorescent antibodies directed against the protein under investigation and of marker proteins for cellular compartments. This is useful to localize the protein under investigation and even more to follow the movements of a particular protein within the living cells. Here we present an example of procaspase-9 movements during the early stages after triggering apoptosis, before its activation can be detected by other biochemical methods.

Apoptosis is a process that controls the number of cells and their quality. Procaspase-9 is the inactive form of one of the main apoptotic initiators, caspase-9. It is activated as a consequence of mitochondrial damage and can be also activated directly or indirectly by other initiator caspases. There were contrasting reports that caspase-9 is in different cellular compartments, i.e. in the cytosol, the nucleus and in the mitochondria. We have determined that procaspase-9 is located in the cytoplasm in physiological conditions in rat neurocrine cells and rat hepatocytes, by transfecting the cells with DNA encoding the fluorescent fusion protein between the caspase-9 and enhanced green fluorescent protein (EGFP) and by immunocytochemistry. However, upon the induction of apoptosis, procaspase-9 is translocated to mitochondria. This shift depends on an activated caspase, other than caspase-9. The colocalization signal of caspase-9 and of mitochondria observed under the confocal microscope does not tell us whether the caspase-9 is associated with mitochondria or it is located closely to the mitochondrial outer membrane. Through biochemical methods, like cellular fractionations, in vitro import of proteins into mitochondria, mitochondrial fractionations and protease treatments of mitochondrial membranes, we determined that procaspase-9 is attached to the outer surface of the mitochondrial outer membrane shortly after the initiation of apoptosis.

MEDCIAL IMAGE ANALYSIS

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Over the last decades, medical imaging has witnessed a diversification of image formation methods, which has led to a rich palette of modalities providing information on many aspects of human anatomy, physiology, and pathology. In order to use the vast amount of available image information efficiently, the relevant image content needs to be extracted, analyzed, and interpreted. For a human operator, it is by no means trivial to interpret the images accurately in a limited amount of time. In addition, such an interpretation is subjective and generally irreproducible. Accordingly, a number of image analysis techniques have been introduced to assist the human expert in a broad variety of tasks, such as image restoration, image segmentation, image registration, motion tracking and change detection, and measurement of anatomical and physiological parameters. Image analysis techniques, which have expanded the role of medical imaging beyond mere visualization, are nowadays used increasingly throughout the clinical track of events, not only within diagnostic settings, but also prominently in the areas of planning, performing, and evaluating surgical and radiotherapeutical procedures.

Oral presentations: abstracts – Predavanja: izvlečki

COMPARATIVE MORPHOFUNCTIONAL ORGANIZATION OF THE ENTERIC NERVOUS SYSTEM IN MAMMALS

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The gastrointestinal (GI) tract fulfils a variety of functions such as transport of luminal content, secretion, absorption of ions, water and nutrients, blood flow, defence against pathogens and elimination of waste material. The enteric division of the autonomic nervous system (i.e., the enteric nervous system (ENS), the brain in the gut, the small brain) organizes and coordinates these activities in a dynamic way through interaction with different cell systems, including the interstitial cells of Cajal, the enteric glia, the smooth musculature, and the vascular, immune and mucosal epithelial systems. The ENS is composed of enteric neurons and glial cells which arise from vagal and sacral precursors cells of the neural crest line. The ENS extends along the entire GI tract and contains an estimated 108 neurons which are situated between two major layers in two interconnected ganglionated plexuses: the myenteric plexus