

P 33 - 7**Studying Single Fluorescing Biomolecules Inside Living Cells Utilizing Various Nonlinear Laser Microscopic Techniques (LSM , FCS , MCS , SMD)**

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Characterization of individual molecular features of single biomolecules (in particular proteins) is of growing importance in both, molecular biology and molecular physiology, due to the increasing impact of individual molecular features in cellular signal transduction. Thanks to the cutting edge sensitivity of optical detectors and photon counting detection schemes single molecule techniques comprise particularly optical methods such as FCS (fluorescence correlation spectroscopy) as well as imaging techniques like CLSM (confocal laser scanning microscopy) and nonlinear laser scanning microscopy (MP-LSM) based on multiphoton excitation processes. Utilizing highly focused laser beams we demonstrate here the feasibility of a commercial LSM (Biorad MRC 1024 extended for FCS and MCS measurements) to image and autocorrelate single fluorescent biomolecules (eGFP-GST) with high spatial resolution and, simultaneously, to monitor photon bursts of single fluorophores with high temporal resolution using a multichannel scaler (MCS, bin width 0.41 ms) in conjunction with the FCS technique. First investigations demonstrate the unique opportunities of multi-modality nonlinear laser microscopy for probing complex biological systems at the single protein level. In our actual approach we utilize our setup to analyze GFP-tagged membrane anchored proteins in cellular plasma membranes.

P 33 - 8**Local proteolytic activity measured by combination of atomic force and fluorescence microscopy**

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Degradation of extracellular matrix (ECM) by local proteolytic activity is a hallmark of tumour cell invasion. As many tumour cell associated proteases are secreted as inactive proenzymes, local activity and not protease concentration is the crucial determinant of ECM proteolysis. Thereby, precise subcellular localization and quantification of proteolytic activity by imaging morphological ECM alteration have proved difficult. A combination of atomic force microscopy (AFM) and fluorescence microscopy (FM) was used to localize and measure proteolytic activity in the microenvironment of highly invasive glioblastoma (U373) cells. AFM is based on the deflection of a fine silicon tip scanning the surface of a sample. It reconstructs a three-dimensional topography of surfaces at a nanometer level. Cover slips were coated with fluorescent ECM proteins (eg collagen IV). ECM fluorescence was reduced by tumor cell induced digestion. FM was used to localize those proteolytic spots and to direct the AFM tip to distinct proteolytic or intact ECM areas. AFM detected a shift in matrix height between intact (9.2nm) and proteolyzed areas (4.1nm) of a gelatin matrix. Taken together, the combination of AFM and FM is a useful tool for studying and measuring local proteolytic processes in cancer cell invasion.

P 33 - 9**Functional quantification of local proteolytic activity in the microenvironment of tumour cells**

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Proteolytic cleavage of extracellular matrix proteins is a typical feature of tumour cell invasion. The proteolytic activity is thereby focused on close proximity of the cells. Here, we describe two assays for functional detection of local proteolytic activity in the microenvironment of living tumour cells. The first assay is based on a thin, fluorescent matrix coating on cover slides. Local digestion of the matrix results in a reduction of fluorescence. The second assay quantifies proteolytic activity of larger cell populations by utilizing the high transepithelial electrical resistance of an epithelial monolayer as an indicator of monolayer integrity. Cell lines and primary cultures of brain tumors and established cell lines of different origins (melanoma, cervix and mamma carcinoma), were compared. Differences in proteolytic activity between tumour entities were demonstrated in both assays. Primary cells of high grade gliomas and invasive cell lines showed different extents of local proteolytic activity and the ability to destroy epithelial monolayer integrity. Low grade primary brain tumours (eg astrocytoma WHOIII) lacked matrix disintegration and consequently disruption of cell monolayers. Taken together, both assays are capable of functional determination of local proteolytic activity and thus are versatile tools for discrimination of high and low invasive tumour cells.

P 33 - 10**Munc-18-1 promotes granule docking in chromaffin cells as revealed by evanescent wave microscopy**

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The presynaptic protein munc-18-1 is an important upstream regulator of synaptic vesicle exocytosis. Its absence leads to impaired docking of large dense-core granules (LDCVs) in adrenal chromaffin cells. In this study we used total internal reflection microscopy to monitor the dynamics of single LDCVs in proximity to the plasma membrane in chromaffin cells from KO and WT mice. The density of LDCVs at the footprint of KO cells was two times smaller than in WT cells or cells rescued by viral expression of munc-18-1. On average LDCVs in KO cells were 'docked' at larger distances to the membrane in line with published electronmicroscopic data. By tracking granular movement in 3D we found that the average excursions from the center position in x, y, and z were the same in KO and WT cells, but were significantly reduced in rescued cells. Granule velocities, however, were higher in KO than in WT or rescued cells, while overexpression of a mutant munc-18-1, which does not interact with syntaxin, only partially rescued the reduced mobility. Low mobility of docked LDCVs in WT was accompanied by periodical (0.5-1s) movements along the z axis, absent in KO cells. This phenotype was rescued with WT but not the mutant munc-18. Taken together, our data indicate that munc-18-1 promotes docking of LDCVs by strengthening tethering forces.