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CELL SURFACE: FROM MOLECULES TO SHAPE

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ORAL PRESENTATIONS

ENVIRONMENTAL STRESS AND AGING AFFECT RECOGNITION OF APOPTOTIC CELLS

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During apoptosis, cell corpes are rapidly internalized by a process involving their recognition and subsequent phagocytosis by engulfing cells. How apoptotic bodies are engulfed is still an argument of debating. Parts of the genetic program for adhesion, uptake, and digestion by phagocytic cells seem to be genetically conserved from *Caernorhabditis elegans* to Primates. A variety of recognition molecules on the surface of cell corpes is important for the recognition and engulfment of the dying cells before membrane lysis. These different *eat me* signals include sugar residues and phosphadytilserine exposure and are fundamental for their recognition by liver cells. In fact, hepatocytes and non parenchymal liver cells can retain and engulf cell corpes with an efficiency that is related to the *mature* apoptotic features. On the other hand, the extent of the exposure of *eat me* signals is a consequence of the intensity of apoptotic stimuli and on the step of the apoptotic program; it is also independent of the cell type, but dependent on the species, cellular aging, apoptotic inducers and on environmental condition (i.e. magnetic pollution) during the apoptotic induction. Magnetic exposure modifies plasma membrane in the presence as well as in the absence of apoptotic inducers; normal cells after a continuative exposure to magnetic fields can be recognized by liver cells at a rate comparable with that of apoptotic ones. Aged cells are more prone to be induced to apoptosis than young ones, but are retained and engulfed by sinusoidal liver cells at a low rate.

HYPERTHERMIA, APOPTOSIS AND ADHESION MECHANISMS IN A NEUROBLASTOMA CELL LINE

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Hyperthermia, used in cancer treatment in conjunction with chemo-radio-therapy, has been recognized as apoptotic trigger controlling both the tumor mass growth and spreading, through the modification of transmembrane proteins and extracellular matrix interactions mediated by integrins. Integrin molecules are formed by two subunits, which bind to ECM components. The integrin complex CD11a/CD18 (LFA-1) plays a key role in cell-cell interactions between telencephalic neurons and microglia. The object of the present work is to investigate the role of LFA-1 in SK-N-MC neuroblastoma cell line and its correlation with apoptosis. SK-N-MC cells were grown in RPMI-1640 as previously reported (1). Hyperthermia treatment was carried out at 43°C for 1 h and 5 h of postincubation at 37°C. For transmission electron microscopy the floating and adherent neuroblastoma cells were processed as previously described (2). Flow cytometry analyses were performed according to the methods detailed elsewhere (3). After hyperthermia treatment, two subpopulation of neuroblastoma cells were found: floating and adherent cells. Ultrastructural morphology characterized the adherent SK-N-MC as elongated cells with large ovoidal nuclei, with prominent nucleoli, whereas floating neuroblastoma cells evidentiated a rounding shape and an apparently smaller nuclear-cytoplasmic ratio. Floating cells express a higher percentage of CD11a positivity than adherent ones (24-60% vs 5-15%, respectively). SK-N-MC cell morphology undergoes relevant changes after hyperthermia, revealing apoptotic features in floating cells, whereas the adherent cells do not seem to enter apoptosis. There is evidence that the treatment is also responsible for $CD11a^+$ cell percentage decreasing from 30% to 16% on floating cells. Taken together, our results suggest that hyperthermia may induce both apoptosis and CD11a expression decrease, even if it seems that these phenomena are not necessarily coupled.

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2.Marini *et al.* Exp. Cell Res. 266, 323 (2001)
3.Luchetti *et al.* Histol. and Histopathol. (2003, in press)

CELL POLARIZATION AND APOPTOSIS IN LEUKOCYTES

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Cell polarization is general feature of cells. It essentially depends on cytoskeleton (mainly on microfilament system and ezrin-radixin-moesin, ERM proteins) and is of great importance in all cell types, neuronal, epithelial or lymphoid cells. In particular, functional state and fate of leukocytes depend on the acquisition of a polarity predisposing lymphocytes either to migration, activation or apoptosis. They have in fact two poles: the leading edge, which deserves as attachment-privileged site where the cell/substrate attachment takes place and the direction of cell movements is established, and, on the opposite side, the uropod, which actually "communicates" in a variety of immune cell activities including activation and apoptosis. Thus, an important requirement for a proper immune response is a transient contact with different substrates, e.g. the extracellular matrix, via the leading edge, as well as a "private" contact both among the immune cells and between immune cells and their targets. Before and during this complex cross-talk between cells and extracellular matrix components, a number of receptors and counterreceptors crowd in the contact sites in order to allow efficient cell-to-cell or cell-substrate interaction. The membrane/cytoskeleton interaction plays a crucial role in tuning these activities and in "predisposing" the immune cells to their function through the acquisition of a polarized phenotype. In particular, on the basis of the literature data, including our results, we suggest that the cell protrusion called uropod, actively bob up and down in the microenvironment as a sensory end, instead of crawling as a "passive" tail of leukocytes. Thus, we propose a role of "antenna-like" ("keraiosome") for this cell projection, and we hypothesize that the keraiosome could be involved in determining the survival or death of leukocytes.

CELL SURFACE MODIFICATIONS FOLLOWING APOPTOSIS IN LYMPHOID CELL LINES

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What changes occur at the apoptotic cell surface and why do they occur is still a controversial matter. They may contribute to the clearance of the dying cell or rather reflect degenerative alterations- becoming eventually a source of autoantigens, and

they may be cell-type specific or not. Prompted by a number of controversial reports, we focused our attention on the oligosaccharide moiety of cell surface molecules, where changes are likely to be reflected by changes in lectin binding. EBV-immortalised B cells were elicited to undergo apoptosis by different stimuli. Results can be thus summarised: 1) radioactive-labelled oligosaccharides were found to be shed from the cell surface at early times after apoptosis induction; 2) the oligosaccharide complement of normal and apoptotic cells, examined by SDS-PAGE of cell lysates followed by binding of a panel of digoxigenin-bound lectins, failed to show qualitative differences between the two cell populations; 3) fluorescence microscopy after exposure to Hoechst 33342 and FITC-lectins showed that a number of lectins, such as ConA, SNA and EC, bound in the same way to normal and apoptotic cells; on the other hand, apoptotic cells had a tendency to loose DSA lectin binding and to concentrate LE binding on surface blebs. Morphological evidences are compared with somehow contrasting results obtained by flow cytometry. We hypothesise that apoptosis-driven cytoskeleton remodelling may result into the confinement of some oligosaccharide-bound molecules to apoptotic bodies. These results are evaluated also in terms of lectin specificity, in order to understand which modifications the oligosaccharide moiety is likely to undergo during lymphoid cell apoptosis.

MAGNETIC FIELDS ALLOW TO DISCRIMINATE TWO DIFFERENT TYPES OF Ca^{2+} INFLUX THROUGH PLASMA MEMBRANE CHANNELS

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Physiological Ca^{2+} influx from the extracellular enviroment occurs through specific channels, and is secondary to Ca^{2+} flux from the endoplasmic reticulum (ER) to the cytosol. This ER flux is elicited in two ways. A), inhibition of Ca^{2+} pumps due to mild stress, causes the passive release of Ca^{2+} in the cytosol, thus causing ER Ca^{2+} depletion and cytosolic Ca^{2+} rise; cells react to ER depletion by triggering an influx from outside, called capacitative Ca^{2+} influx, thus replenishing the stores. B), membrane receptors stimulation elicits a signal tranduction that stimulates the opening of ER Ca^{2+} channels, and the consequent ER flux. Also here, cells react to ER depletion by triggering capacitative influx; however, in these instances cytosolic Ca^{2+} rise has a precise meaning, since it drives the downstream signaling process: to this purpose, an additional Ca^{2+} influx is elicited, through different channels, known as non-capacitative Ca^{2+} influx. We report here that a 60 Gauss static magnetic field, obtained with a magnet of known intensity, allows to discriminate two different types of Ca^{2+} influx: it increases Ca^{2+} influx consequent to ER Ca^{2+} pumps inhibition, i.e., with thapsigargin (Fanelli et al., 1999, FASEB J. 13, 95-102). In contrast, it attenuates the influx due to stimulation of melatonin plasma membrane receptors. This attenuation abolishes the anti-apoptotic effect of melatonin, probably by aborting the downstream signal transduction.

DIFFUSIVE CELLULAR PROCESSES FROM MICROSCOPIC TO MOLECULAR LEVEL USING ONE- AND TWO-PHOTON EXCITATION FLUORESCENCE

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Microscopical and spectroscopical techniques, utilizing steady-state and time resolved one- and two-photon excitation of fluorescence, are useful biophysical tools for the study of cellular processes. Information related to cellular functioning and diffusive processes can be obtained by using complementary data achievable by means of confocal and two-photon imaging, single molecule detection (SMD), fluorescence lifetime (FL), Forster fluorescence resonance energy transfer (FRET), fluorescence correlation spectroscopy (FCS), fluorescence recovery after photobleaching (FRAP) and fluorescence loss in photobleaching (FLIP). Both the microscopic and the molecular level can be addressed towards single molecule dynamics in complex systems like the biological cell. One lacuna of the studies related to bio-molecules trafficking in and out cellular systems is given by the gap between observations in highly concentrated or crowded environments and the behaviour of single bio-molecules studied in solution or in bulk systems. A comparatively new colloidal system, named nanocaspule, can fill the gap. In fact, nanocapsules constitute a novel type of fuzzy nano-organized polyelectrolyte shells being able to confine macromolecules in a controlled three-dimensional nano- and micro-environment, and to mimic molecular crowding in cellular environments. Coupling the above mentioned techniques with nanocapsule technology allows to gain further insights in the cellular diffusive processes.

FROM SINGLE MOLECULE MECHANICS AND KINETICS TO CELLULAR ADHESION

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The investigation of biomolecules with respect of their structure and their molecular interplay is a central issue in life sciences. This requires an interdisciplinary approach from biology, chemistry and physics in order to adequately prepare the molecules of interest and to address them with dedicated techniques. Modern single molecule (SM) technologies, such as atomic force microscopy & spectroscopy (AFM/S) or laser tweezers, allow observation, probing and manipulation of individual biomolecules, molecular complexes and aggregates at sub-nanometer spatial resolution and sub-piconewton force sensitivity. The fundamentals of these methods will be summarized by presenting single molecule binding experiments between antigens and mutated antibody fragments [1,2], small ligands and DNA [3], a transcriptional regulators and DNA [4]. Furthermore, the application of these SM-Methods to CAM (cell adhesion molecules) like adhesion proteoglycans in marine sponges [5] and the leucocyte recruitment on activated endothelium (P-selectin-PSGL-1) [6] will be presented and discussed.

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THE FRACTAL FORM OF CELL SURFACE

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The functional integrity of the plasma membrane expresses through the complex organization of its own morphoultrastructural constituents. Their spatial redistribution may not only reveal but precede the occurrence of cellular processes either physiologic or pathologic, particularly at the onset stage when they fail to be fully expressed or to be detectable by conventional methods. By adopting tools inspired by the fractal geometry [B. Mandelbrot], developed for natural elements and biological components mostly irregular and self-similar at different scales of inspection, it is possible to accurately measure the fractal dimension (FD) of fine morphological changes which take place on plasma membranes and other cellular organelles, as visualized from electron microscopic pictures [G. A. Losa, Biology Forum 95, 2002]. Blasts from leukaemia patients had a plasma membrane with a profile smoother (smaller FD) than that found for the plasma membrane of normal immunocompetent T and B blood lymphocytes, likely due to an intense membrane shedding. A loss of structural complexity of plasma membrane and selected nuclear domains (reduced FD) was measured in human breast cancer cells at the initial stage of apoptosis. In the presence of steroid 17-β-estradiol, a stimulator of cell proliferation, breast cancer cells showed a structural pattern of membrane-bound heterochromatin more irregular (increased FD) than in cells cultured with an antiproliferative glucorticoid. Viable cat oocytes had a cell surface more complex than that found in non viable elements. In conclusion, the fractal geometry allows structural properties and form changes to be examined in connection with dynamic functional and pathological events arising at the cell surface, in the nucleus and other cellular organelles.

COLOCALIZATION ANALYSIS OF THE PRION PROTEIN AND CAVEOLIN 1 IN CONFOCAL MICROSCOPY.

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Comprehension of the mechanisms used by the surface cell membrane to transduce and amplify extracellular signals has advanced rapidly in the past few years thanks to the identification of novel signal transducers and to new concepts of the plasma membrane organization. In particular, it has been proposed that cholesterol and sfingolipids segregate in liquid-ordered phases – called rafts - where proteins involved in signal transduction get dynamically organized (Brown and London, 2000). A subdomain of lipid rafts are the flask-shaped plasma membrane invaginations named caveolae. The caveolae are implicated in several cell functions, including endocytosis and transport of cholesterol and proteins. Caveolins seem to play a crucial role in the caveolae organization. Based on the ability of caveolins to organize and concentrate signalling molecules, caveolae may also serve in signal transduction (Parton, 1996). Similar to other GPI-anchored proteins, PrP^c inserts into rafts. A misfolded isoform of PrP^c, termed PrP^{Sc}, is believed to cause prion diseases that affect humans and animals (Prusiner, 1991). The physiologic function of PrP^c is still unknown. PrP^c may play a role in the transport and/or homeostasis of Cu^{2+} (Brown, 1999), with neuroprotective functions (Bounhar et al., 2001). We studied and expanded the proposed functional coupling between the cellular prion protein PrP^c and caveolin 1. To this end, we used PrP transfected GN11 murine immature neurons that naturally express abundant cav1. By confocal microscopy co-localization analysis we demonstrate that the two proteins assemble at the plasma membrane under a variety of conditions, in particular after cell stimulation by antibody ligation of PrP^c in the presence of physiological Cu^{2+} amounts.

THE INTERACTION OF A SINGLE MOLECULE OF FIBRONECTIN WITH A LIVING BACTERIUM

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The binding and unbinding of fibronectin to a single living Staphylococcus epidermidis bacterium was reproduced in physiological conditions and at the level of single molecules. The specificity of this interaction was demonstrated. The energy landscape along the binding and unbinding pathway was mapped and the dynamic association and dissociation rate constants were determined. An inner potential well of the binding mode was identified. The dynamic features and the dependence of the binding probability on the contact time between fibronectin and a single bacterium were discussed and compared with those reported for other adhesion molecules that mediate different dynamic states of adhesion of a cell under a hydrodynamic flow.

ULTRASTRUCTURAL AND SPECTROSCOPIC METHODS IN THE STUDY OF DRUG-MEMBRANE INTERACTION

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The plasma membrane is an important target of the mechanism of action for many anticancer drugs. In this study, we investigated the interaction of anthracycline antibiotics with the plasma membrane of human erythrocytes by morphological, ultrastructural, microanalytical and spectroscopic methods. Scanning and transmission electron microscopy analysis indicated that doxorubicin (DOX) induced dose-dependent alterations of the cell morphology and plasma membrane ultrastructure. In particular, the treatment with 50 μM DOX converted most cells to stomatocytes and at higher concentration (100 μM) the cell surface underwent a remarkable modification, assuming a mottled appearance. The freeze-fracture method revealed a very unusual distribution of the protein intramembrane particles (IMPs) on both fracture faces of the plasma membrane of erythrocytes trated with DOX. The formation of numerous particle-free domains of variable geometry was detectable suggesting that the DOX molecules were incorporated within the lipid bilayer. Electron energy loss spectroscopy (EELS) revealed a reduction in the P/C ratio in treat-

ed membranes, probably due to a phospholipid "dilution" following the incorporation of DOX molecules. The radiowave dielectric spectroscopy indicated a decreasing in membrane conductivity suggesting that the interaction of the drug with the membrane lipids could affect the function of specific ion channels. The results obtained allowed us to propose a structural model of the DOX-membrane interaction in which drug molecules self-associate in the phospholipid bilayer inducing remarkable changes in the structural and functional properties of the plasma membrane. In conclusion, these observations seem to strengthen the hypothesis that the anthracycline antibiotics can also exert their cytotoxic action at the membrane level.

DRUG RESISTANCE MECHANISMS IN TUMOR CELLS

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Multidrug resistance (MDR) is the major obstacle in the pharmacological treatment of tumors and infectious diseases. MDR is a multifactorial phenomenon that involves elevated expression of drug transporters as well as additional biochemical changes that contribute to the drug resistant phenotype. Among drug transporters, the P-glycoprotein (P-gp), encoded by MDR1 gene in humans, is an integral plasma membrane protein, capable of transporting several chemically unrelated compounds. Its overexpression on the plasma membrane of tumor cells is often responsible of neoplasm unresponsiveness to clinical pharmacological treatment, mainly due to a decrease of intracellular drug amount. Moreover, P-gp seems to have a drug-independent role in the inhibition of *in vitro* apoptosis, and to associate with the actin cytoskeleton trhough ezrin, radixin and moesin. Recently, interest has been focused on the possible relationship between drug resistance and cancer invasion and metastasis. In a resistant variant human melanoma cell line (M14 ADR) we found that overexpression of P-gp co-variates with a phenotype of elevated *in vitro* invasion. Furthermore, we studied the expression of P-gp in relation with that of CD44 molecules, the major cell surface receptor for hyaluronate implicated in cell adhesion, metastasis and tumor progression of melanoma cells. The obtained results suggest that P-gp and CD44 might co-operate to confer a more invasive and resistant phenotype. Recently, the phenomenon of MDR has been reinterpreted on the basis of the recently proposed concept of microvillar signalling: the microvillar surface organization is lost in rapidly growing cells such as tumors but is restored during exposure to cytotoxins by induction of a prolonged Go/G1 resting phase.

MORPHOFUNCTIONAL STUDIES ON HUMAN SALIVARY GLANDS STIMULATED *IN VITRO* BY RECEPTOR-SPECIFIC SECRETAGOGUE DRUGS

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To visualize the events occurring during secretion we have set up an incubation method of samples of human salivary glands in a new inorganic medium that allows not only their morphological study, but also the biochemical analysis of the salivary peptides secreted into it. Results concerning the latter point are not reported here. One mm^3 pieces of normal major and minor salivary glands were incubated for 30' in an inorganic medium containing 1 µM D,L Isoproterenol, 1-20 µM carbachol, 10 µM mm Clozapine, or 10 µM SNI-2011. In many cases, the specific inhibitors (propranolol for ß-adrenergic receptors, atropine for muscarinic receptors, phentolamine for α-adrenergic receptors), were added, while media lacking the secretagogue served as controls. After incubation, specimens were treated for LM and TEM, or for our OsO_4 maceration method for HRSEM. In mucous cells of all glands mucous droplets are released by muscarinic agents, while no stimulation is seen with the ß-adrenergic agonist Isoproterenol. Cytological events during mucus discharge are similar, in all human glands, to those described by TEM on stimulated mucous cells of subligual glands of rodents. Moreover, even if human minor glands have a prominent cholinergic supply with scanty adrenergic nerves, the response of serous/seromucous cells, particularly evident with HRSEM at the intercellular canaliculi, is similar in all glands. It seems very likely that serous/ seromucous, and mucous cells of major and minor glands respond, in man, to the same receptors.

ANTI-HYPERTENSIVE CALCIUM CHANNEL ANTAGONIST DRUGS: PRELIMINARY STUDIES ON ERYTHROCYTES FROM NORMAL SUBJECTS AND THERAPY CANDIDATES

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Erythrocyte morphology has been already studied in essential hypertension (EH) and cell membrane alterations have been observed. Although relationships among red cell rheological, biochemical and morphological properties still appear complex and not clearly understood, workers agree that uncontrolled loading of ions in the cytosol impairs membrane deformability and induces erythrocyte shape changes. In this study we have performed: i) an "ex vivo" morphological examination of erythrocytes obtained from three groups of subjects, namely healthy, healthy with familial EH and EH suffering subjects; ii) an "*in vitro*" exposure of normal erythrocytes to different calcium channel antagonist drugs commonly used in EH treatment (Diltiazem, Nimodipine, Nifedipine and Verapamil), followed by morphological examination and intracellular ATP and GSH concentration assays. In both cases erythrocyte morphology study was carried out by using the novel automated method we have recently published [Albertini et al., Cytometry, 52A, 12, 2003]. Our results show that, in comparison with controls, morphological modifications are evident both in erythrocytes from EH and from healthy with familial EH subjects. In the "*in vitro*" experiments little morphological modifications and variations in ATP concentrations are only observed in erythrocytes incubated with Diltiazem and Verapamil. The latter results distinguish the two drugs from Nimodipine and Nifedipine which are both dihydropyridines. So, our preliminary findings indicate that EH may be predicted by erythrocyte morphology changes which may be prevented by drugs inhibiting calcium fluxes which, in turn, may delay uncontrolled accumulation of calcium without influencing the oxidative state of the cell.

ADULT STRUCTURAL PLASTICITY AND NEUROGENESIS IN THE MAMMALIAN OLFACTORY SYSTEM

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Continuous structural plasticity and neurogenesis in the adult mammalian central nervous system have been clearly established since a decade. The analysis of such plasticity can throw some light on processes of primary neural differentiation, but also on the specificities of the adult tissue, imposing some severe limitation to brain repair in adulthood. The olfactory system, both in the periphery (olfactory neuroepithelium) and in the brain (olfactory bulb), is a major site of adult neurogenesis. Structural plasticity in this system displays features which are absent in the hippocampus, the other main neurogenetic area of the adult brain. The olfactory receptor neurons are unique since they send axonal processes enwrapped by ensheathing glial cells that make them capable to enter the mature central nervous system. The olfactory bulb is the brain region most strikingly enriched throughout life by new neurons, whose precursors are generated by neural stem cells residing into the forebrain subependymal layer and then undergo long distance cell migration. Recently, new cues arised from the study of growth factors and their receptors on migration and differentiation of newly formed cells as well as on the neuroanatomic make-up, which warrantees a niche for neural stem cells.

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ROLE OF SPECIFIC PROTEINS IN THE PATHOGENESIS OF BASAL GANGLIA DEGENERATIVE AND REGENERATIVE PROCESSES

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The ubiquitin-proteasome (UP) system is an ubiquitary, multienzymatic proteolytic pathway, which removes misfolded proteins deriving from plasma membrane including internalized receptor. Recently, mutations of genes encoding for proteins of the UP were found to be responsible for inherited forms of Parkinson's disease (PD). These mutations involve three known gene products: α-synuclein (PARK 1), parkin and ubiquitin-C-hydrolase-L1. In keeping with this, intracellular inclusions known as Lewy bodies (LB), a pathological hallmark of sporadic PD, stain specifically for proteins associated with UP including α-synuclein, Uch-L1 and parkin, an E3 ubiquitin-protein ligase. This indicates that a failure of this multi-enzymatic complex which clears misfolded proteins from the plasma membrane might represent a final common pathway in the pathogenesis of PD. The role of specific proteins belonging to the plasma membrane was investigated during inhibition the UP system as well as during recovery processes. Uptake of various neurotoxins by the plasma membrane dopamine transporter (DAT) induces striatal dopamine (DA) loss accompanied by a damage to striatal nerve endings arising from the substantia nigra. On the other hand, inhibition of the UP system produces multi-membrane neuronal inclusions containing α-synuclein, ubiquitin, and several components of the UP system. Cell inclusions occur also following amphetamine derivatives. To assess whether formation of UP-positive neuronal inclusions might represent a crucial aspect of biochemical mechanisms underlying METH toxicity, we observed by transmission electron microscopy the effects of *in vivo* and *in vitro* treatment with METH. We found a tight correlation between intracellular inclusions and DA metabolism. Immunocytochemical investigation showed that these METH-induced cytosolic inclusions stained for ubiquitin, α-synuclein and UP-related molecules.

SIGNAL MOLECULES AND RECEPTORS IN NEURONAL DIFFERENTIATION

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During development, neural circuits are regulated by signal molecules and by the combination of different receptor subunits which, sometimes transiently, appear in precise phases of morphogenesis, also in relation to the different location of their targets. Among the morphogens playing a fundamental role in developing cerebellum, the amino acids glutamate and GABA (the main excitatory and inhibitory neurotransmitter, respectively) are inextricably linked in the control of neuronal excitability, their signaling role being exerted through the binding to cell surface receptors. Differentiation of Purkinje cells deserves particular interest: in fact, these neurons reflect the patterning of local circuits and the achievement of cellular connectivity, being the sole output from the cerebellum; moreover, Purkinje neurons receive both excitatory and inhibitory synapses. The distribution, interaction and role of ionotropic and metabotropic receptors of glutamate and GABA have been investigated by immunocytochemical techniques, in bright-field and fluorescence microscopy, during Purkinje cell differentiation in crucial steps of cerebellar cortex maturation in the rat. A single injection of the cytostatic cisplatin was used as an experimental mild injury; this was administered in a vulnerability window of the neocerebellar vermis, to identify receptor changes in the damaged neurons and to analyse the regenerative capabilities of the neural tissue. Special attention was paid to some receptors marking molecular and structural changes in the reorganization of cerebellar cytoarchitecture after injury. The phenotypic changes of Purkinje cell dendrites were not paralleled by the usual patterns of excitatory and inhibitory synapses. In order to get a deeper insights into the functional role of glutamate and GABA receptors in neural plasticity, additional parameters concerning cell proliferation and survival, migration, synaptogenesis were also considered.

STRUCTURAL PLASTICITY OF ADULT RAT DENTATE GYRUS: VITAMIN E ROLE

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New granule cells are continually generated in the adult rat dentate gyrus (DG). In previous works we found that vitamin E is an exogenous factor regulating different steps of adult DG neurogenesis. In particular we demonstrated that in rats supplemented by α-tocopherol, the most important compound of vitamin E, cell proliferation was inhibited and cell death, responsible for the elimination of a part of proliferated cells, was reduced. As the new neuron survival is enhanced, our hypothesis is that an accumulation of new neurons occurs after α-tocopherol supplementation. Since the immature neuronal marker expression is helpful in detecting newly generated granule cells, in the present study qualitative and quantitative analysis of molecular markers such as PSA-NCAM, TUC 4, and DCX was performed by means of immunohistochemistry in the DG of

adult rats after 6 weeks of subcutaneous α-tocopherol injection. Alpha-tocopherol levels significantly increased both in the plasma and in the brain after supplementation. The immature neuron marker-positive cells significantly increased and the dendrites labelled for PSA-NCAM greatly extended to the molecular layer in the a-tocopherol treated rats. The increased number of expressing neuron marker cells may be regarded both as an increase in the number of the new cells migrating and forming synapses or as an increase in the marker expression by mature neurons remodelling their synapses. These results confirm that vitamin E regulates adult neurogenesis and demonstrate that it promotes a structural remodelling in DG.

MORPHO-FUNCTIONAL DIFFERENTIATION OF GRANULE CELLS OF ADULT RAT DENTATE GYRUS

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New neurons originate from proliferating precursors throughout adult life in mammal dentate gyrus. A number of daughter cells precociously die, while the surviving cells differentiate into neurons within granule cell layer (GCL). This imply that neurons at different stages of morphological and functional differentiation must be found in adult dentate gyrus. New neuron survival is positively or negatively affected by learning, depending on their differentiation stage. Recently the morpho-functional differentiation of new neurons in the adult dentate gyrus aroused new interest because of demonstration that they are necessary for some kinds of learning during their differentiation. This finding suggests that immature neurons have morphological and functional features making them particularly suitable for coding some kinds of memory. A comparative morphological and functional study of neurons has been carried out in rat GCL after the anatomical development. Single cells was electrophysiologically characterized: membrane ion currents were determined and membrane passive properties were measured to infer information about cell surface area; synaptic input was demonstrated and characterized. The same cell was filled with biocitine to demonstrate the whole structure of the dendritic tree. Afterwards the same cell so identified was immunoistochemically characterized as immature or mature neurons. The following cell classes were found: 1. Small cells with several primary dendrites, showing no synaptic input and showing voltage-dependent K^+ current, and sometimes voltage-dependent Na^+ current. 2. Cells with a dendritic tree extending within GCL, showing GABA (excitatory) synaptic input and often voltage-dependent Na^+ and Ca^{2+} current. 3. Cells with a dendritic tree extending into molecular layer, showing GABA and glutamate synaptic input and often voltage-dependent Na^+ and Ca^{2+} current. 4. Cells with a complex dendritic tree extending into molecular layer, showing GABA (inhibitory) and glutamate synaptic input and voltage-dependent Na^+ current.

A MECHANOCHEMICAL APPROACH TO THE ROLE OF HUMAN ANGIOSTATIN IN ANGIOGENESIS.

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The Single Molecule Force Spectroscopy (SMFS) methodology makes it possible to investigate the forces that hold together the structure of proteins and to characterize the mechanical role of those proteins that in-vivo are involved in transport and mechanochemical processes. Human angiostatin is a 57kDa proteolitic fragment of human plasminogen. Angiostatin inhibits angiogenesis by interfering with integrin mediated endothelial cell adhesion and migration. Angiostatin is thus involved in mechanically regulated signaling pathways and is expected to experience mechanical force *in vivo*. Angiostatin consists of five compact globular modules called Kringle domains that are built around a hydrophobic core and exhibit a triple-loop topology defined by three internal disulfide bonds. These disulfide bridges limit the unfolding and therefore the degree of molecular extension of the single domains under a mechanical stress. Angiostatin can thus act as a mechanical switch only whenever the stress is coupled to a modulation of the redox conditions of the environment. The control of the topology and of the mechanical properties of angiostatin by the extent of pairing of the internal disulfide bonds was studied by the SMFS methodology. This methodology makes it possible to evaluate, at the single molecule level, the distributions of the thiol/disulfide intermediates obtained under a mechanical stress in different reducing conditions (1). Under this basis a mechanochemical approach to the role of angiostatin in angiogenesis can be proposed.

(1) Y. Bustanji, B. Samorì, Angew. Chemie Int. Ed. 114, 1616-1618 (2002).

THREE DIMENSIONAL ARCHITECTURE OF MICROVESSEL STUDIED BY CORROSION CASTING TECHNIQUE

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Corrosion casting technique consists of a low viscosity acrylic polymer injection in the vascular beds of many organs. The injected medium (MERCOX), thank to a specific catalyst, hardens inside the vessels, faithfully reproducing their internal shape. The cast of vessels is then revealed removing all tissues around, with a quite long immersion in hot alkali or acids, becoming visible in all its branching patterns until the thinnest capillary sections by scanning electron microscope analysis.

We applied this method to some organs: in the *stomach* we were able to visualize the disposition of capillaries around mucosal glands and in the muscular layer. In the *kidney* the most impressive result was the accuracy of this technique to reveal cellular pores of endothelial cells and the sharp three dimensional impression of their membrane's extensions. In the *brain* the corrosion casting method allowed us to study the entire architecture of cerebral vessels starting from pial capillaries, till cortical and subcortical ones, also describing the plexiform vascular architectures of dura mater and choroideal plexuses. Applied to the human digit corrosion casting technique revealed essential to describe the angiogenic buttons in the nail root along with all dermal corpuscles associated to thermoreceptorial functions. Moreover it was possible to follow a clearly shaped vascular fingerprint exactly corresponding to the cutaneous one. We also came across a few artefacts mostly due to the injective injury occurred during perfusion. Although the corrosion casting technique requires a quite easy protocol, also thank to the modern technology, it needs specific technical variations for each organ studied.

REGULATORY MECHANISMS OF ANGIOGENESIS

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Angiogenesis, the growth of new capillary blood vessels, occurs both in physiological conditions (wound repair and, cyclically, in female genital system) and pathological conditions such as chronic inflammations and tumors. It develops through several steps including basement membrane degradation by proteolytic enzymes secreted by the endothelial cells, chemotaxis toward the stimulus and proliferation of these cells, canalization, branching and formation of vascular loops, stabilization and functional maturation of neovessels following perivascular apposition of pericytes and smooth muscle cells, neosynthesis of basement membrane constituents. Under physiological conditions, angiogenesis is dependent on the balance of positive and negative angiogenic modulators within the vascular microenvironment, and requires the functional activities of a number of molecules, including angiogenic factors, extracellular matrix proteins and proteolytic enzymes. As a consequence, angiogenic endothelial cells have a distinct gene expression pattern that is characterized by a switch towards an invasive phenotype. Tumor angiogenesis is regulated by several factors, mainly growth factors for the endothelial cells, secreted by both the tumor and host inflammatory cells, and mobilized from extracellular matrix stores by proteases secreted by tumor cells. Regulatory factors also include the extracellular matrix components and endothelial cell integrins, hypoxia, oncogenes and tumor suppressor genes. Angiogenesis is mandatory in the process of tumor progression (growth, invasion and metastasis), since it conveys oxygen and metabolites, whereas endothelial cells secrete growth factors for tumor cells and a variety of proteinases which facilitate invasion, and increase opportunities for tumor cells to enter the circulation.

THE MICROVASCULAR NETWORK: ULTRASTRUCTURAL AND MORPHO-FUNCTIONAL ASPECTS

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In the last years new techniques allow to obtain resin vascular casts (vascular corrosion casts, vcc) to be observed by Scanning Electron Microscopy (SEM); these allow a high resolution and a detailed three-dimensional study of the microvascular bed. The cast preparation considers the following steps:

A) pre-casting procedure: 1) after identification of the blood vessel that is suitable to be perfused according to the target organ, the vessel is cannulated and the blood is washed out thoroughly with physiological saline at r.t. with or without anticoagulants, vasodilatory and spasmolitytic agents; 2) possible perfusion fixation with gluteraldehyde; 3) identification of the perfusion pressure suitable to obtain a good quality cast. B) casting procedure: 1)injection of enough resin to obtain complete filling of microvessels; 2) resin polymerize within 24 h at r.t.; 3) complete or partial dissection of the target organ/tissue. C) tissue corrosion: 1) after microdissection under the dissecting microscope, the injected specimens are exposed to maceration in alkalis or acids in order to obtain a cast that is free of tissue remnants; 2) corrosion could be complete or partial to better study perivascular structures; 3) cast washing and cleaning with H_2O or CCl_3COOH 5%. D) Drying: specimens are immersed in distilled water, frozen at -20°C and dried. E) Mounting: casts are mounted on metal stubs with conductive mounting media. F) Conductive treatment: casts are coated with metal or gold by high energy sputtering. G) SEM observation. The characteristic endothelial imprint on the cast surface allows the discrimination of arteries and veins and then the localization of the afferent and efferent vessels. Moreover, it is possible to obtain different morphometric data (e.g. vascular density, diameter, length, etc) of the whole microvascular network. Vcc observed by SEM seem to be a fundamental complement not only in the study of the fine microvasculature of normal organs but also in study of morphofunctional aspects of microcirculation under experimental or pathological conditions.

INTRACAVITARY PLEURAL CANCER CELL DISSEMINATION

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Vascular endothelial growth Factors (VEGF) and its receptors can directly influence the microenvironment of pleural and pericardial cavities. Results will be presented on the autocrine/paracrine loops recently discovered in malignant mesothelioma (MM) cells and on the regulatory molecular pathways underlyning their functions. Avascular role of pro-angiogenic growth factors and their implication in cancer cell dissemination into mesothelial cavities will be discussed. An immuno-suppressive environment may represent a key advantage for tumor progression. Moreover, opportunistic viral replication and infection may occur. Recent data from ours and others will be presented to describe intrapleural immunomodulatory events. Experimental models of tumor cell spreading into pleural and peritoneal cavities will be also presented. Finally, the potential implication in early diagnosis, prognosis and experimental therapy of these findings will be overviewed.

CLINICAL RELEVANCE OF TUMOR DIFFUSION CELLULAR MOLECULAR MARKERS

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The process of tumor metastasis (TM) involves several interplayed molecular mechanisms which schematically are responsible for the four main steps: impairment of cell adhesion, proteolysis, migration and angiogenesis. *E-cadherin*, matrix metalloproteinases (*MMPs*), urokinase plasminogen activator system (*uPA*) and vascular endothelial growth factor (*VEGF*) have shown an influence on the natural history of the disease and are among the novel molecular markers which are involved in the different aspects of TM. *E-cadherin*, a cell adesion molecule, seems to play a major role and its codifying gene (*CDH1*) is now considered a genuine tumor suppressor gene. A dramatic loss of adhesion is found in tumors without its normal expression on the cell surface. *MMPs* are zinc dependent proteolytic enzymes which cleave extracellular matrix (ECM) proteins as well as non-matrix substrates (growth factors, cell surface receptors, etc). They seem involved in many steps of TM, but invasion seems the most important. *uPA* is a serine protease capable to initiate an extracellular cascade of proteolysis involving the activation of plasminogen and *MMPs*. This cascade remodel ECM and basement membrane, allowing for the movement of cells across and through these barriers. Processing and releasing of various growth and differentiation factors also contribute to the evolution of a migratory or invasive cell phenotype. uPA has been shown to be required for the movement of

cells through a matrix and its system appears to be central for the migration of endothelial cells during the process of angiogenesis. *VEGF* plays a major role in angiogenesis which, when pathological, contributes to the development of numerous types of tumors and metastasis formations. Nowadays it is widely accepted that the transition from the latent to the invasive and metastatic phase of a cancer is linked to the angiogenic switch. In vivo, there is growing evidence of the prognostic role of the reported markers in patients with common solid neoplasms (i.e. gastric, colorectal, breast and lung cancer). Novel therapeutic agents whose mechanism of action is the restoration of adhesion, when this function is impaired in the case of loss of *E-cadherin* or the abrogation of *VEGF* when angiogenesis is stimulated, are under investigation in clinical trials.

ROLE OF CD99 IN THE TUMORIGENESIS AND METASTATIZATION OF SARCOMA CELLS

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CD99 gene is broadly expressed on many human cell types with a particular strong expression on lymphocytes and, among malignancies, on Ewing's sarcoma. Triggering of CD99 membrane protein has been known to be involved in cell migration, adhesion and apoptosis. Ligand of CD99 is still unknown and the biological role of this molecule, in its unbound form, is still obscure. In an effort to highlight whether the high expression of CD99 molecule in Ewing's sarcoma cells represents a reminiscence of its origin or rather gives an advantage in Ewing's sarcoma progression, we transfected the CD99 cDNA into a human sarcoma cell line that was negative for the expression of this antigen. Both the two forms of CD99 were transfected: the long, wild type CD99 (wtCD99) and its trucated variants (sCD99). *In vitro*, the two forms induced opposite effects with respect to growth in semisolid medium and migration. In particular, wtCD99 transfected cells displayed lower growth ability in soft-agar and decreased cell motility and invasive ability than parental cells, whereas sCD99 expressing cells gave an higher number of colonies in soft-agar, a significantly higher migration and a reduced homotypic adhesion. Moreover, whereas no alterations were observed in the intracellular MAPK-mediated signalling pathway of these cells,wt CD99 transfected cells showed significantly reduced levels of phosphorylated AKT, a member of the PI 3-K pathway that is generally important for survival, cell migration and adhesion. In vivo, the expression of wtCD99 completely abrogated the ability of U-2 OS cells to give lung metastasis in nude mice, whereas sCD99 induced enhancement of metastatic ability. Taken together these findings indicate that CD99 appears as a primitive marker of the undifferentiated Ewing's sarcoma cells, rather than a molecule involved in its progression, and further support the view of this antigen as a promising entry for therapeutic intervention in Ewing's sarcoma patients.

POSTERS

MORPHOLOGICAL AND BIOCHEMICAL ANTIOXIDANT EFFECTS OF RHODIOLA ROSEA

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The extracts of roots and rhizomes of *Rhodiola rosea L.* (Crassulaceae) are rich in antioxidant substances as p-tyrosol, organic acids and flavonoids. This suggests a possible therapeutic actions and it has become a component of many dietary supplements. In order to test the antioxidant capacity of *Rhodiola r.* exctract, the model of human erythrocytes (RBC) exposed to hypochlorous acid (HOCl) was utilized, being HOCl a powerful natural oxidant generated by activated phagocytes with wide biological targets. To perform the study, water-soluble fraction of *Rhodiola r.* hydro-alcoholic dried extract was added to RBC (10% v/v) incubated with 0.5 mM HOCl in the presence or not of aqueous extract of *Rhodiola r.* (10-200 µl/100 µL RBC). After incubation, RBC were treated for determination of GSH, GAPDH and hemolysis. The SDS-PAGE was performed according to Laemmli's method. Treated and control erythrocytes were standard prepared for the scanning electron microscope (SEM) and observed at 15 kV. Our results demonstrated that *Rhodiola r.* extract is able to protect, in a dose dependent manner, human erythrocytes from GSH depletion, GAPDH inactivation and was able to hold up hemolysis induced by HOCl, whereas the SDS-PAGE patterns of erythrocyte membrane proteins evidenced the presence of high molecular weight clusters with a binding of hemoglobin. The ultrastructural analysis clearly evidenced the dose dependent role of *Rhodiola r.* in RBC preservation till a maximum of 150 µl extract/100 µl RBC: the addition of more extract, in fact, seems to cause the loss of typical biconcave shape of the erythrocytes and a shrinkage of cell membranes. Taken together, our data are able to confirm the antioxidant role of *Rhodiola r.* but, at the same time, they suggest a possible toxicity at high concentration of the extract.

EFFECTS OF ESTROGENS ON INVERTEBRATE IMMUNOCYTE MORPHOLOGY AND FUNCTION

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It is now well known that natural and environmental estrogenic compounds markedly influence the mammalian immune system. In bivalve molluscs, such as the mussel *Mytilus galloprovincialis* Lam, circulating hemocytes, that resemble the monocyte/macrophage lineage, are responsible for innate immunity. In this work the effects of the natural estrogen 17β-estradiol (E_2) on mussel hemocytes were evaluated and the results were compared with those of the synthetic estrogen diethylstilbestrol (DES). The results indicate that nM concentrations of E_2 induced progressive changes in cell morphology, as evaluated by SEM; the most evident effects were a general decrease in the number and length of membrane extensions and an apparent release of extracellular material. The E_2-induced

cell changes were inhibited by the classical antiestrogen Tamoxifen, indicating that activation of estrogen receptors are involved in the effects of E_2. Moreover, E_2 caused a concentration-dependent decrease in lysosomal membrane stability that was prevented by Tamoxifen. Western Blot analysis of hemocyte protein extracts show that E_2 also affected the phosphorylation state of components of both the MAPK (Mitogen Activated Protein Kinase) and STAT (Signal transducers and Activators of Transcription) families. DES mimicked the effects of E_2 at concentrations 1000 times higher (μM) than those of the natural estrogen. Overall, our data demonstrate that in invertebrate cells both natural and synthetic estrogens can affect cell morphology and function.

CD11a EXPRESSION ON DIFFERENT CD34 SUBSETS FROM CORD BLOOD

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Cord blood (CB) has proved itself an excellent source of stem cells for related and unrelated transplants (Migliaccio AR et al., Ann. Ist. Super Sanità, 2001). Stem/progenitor/cells are usually selected on the basis of CD34 surface expression. Recent studies have demontrated the importance not only of the absolute number of $CD34^+$ cells re-infused but also their state of commitment for a successful engraftment (Xiao M et al., Exp Hematol,1999). To this regard, molecules such as CD71, (late erythroid marker), CD90 and CD135 (early expressed), combined with CD34 are becoming of scientific and clinical interest for CD34 subsetting and to predict a successful engraftment. Moreover, modulation of adhesion molecule expression has been demonstrated to be involved in the mobilization and engraftment of haematopoietic cells (Orschell-Traycoff CM et al., Blood, 2000). In the present study, the expression of the adhesion molecule CD11a combined with CD71, CD90, and CD135 antigens (defining CD34 subsets) was evaluated by flow cytometry within the $CD34^+$ cells from CB specimens. Our results indicate that CD11a was expressed on about 60% of $CD34^+$ cells in CB samples analysed. Within CD34 and CD11a double positive cells, the percentages of cells expressing CD90 and CD71 were 4,8 (±2,5) and 6,6 (±3,0), respectively. CD135 expression was extremely variable (ranging from 0,3% to 2%), according to our previous data on CD135 distribution in the entire $CD34^+$ stem/progenitor cells. Our preliminary findings indicate that within $CD34^+$ CB cells, CD11a is expressed on different subsets independently on their state of commitment. Further analyses need to be performed to better delineate the CD11a pattern of expression within $CD34^+$ subsets.

RELEASE OF FIBRONECTIN FROM ACTIVATED PLATELETS AND FORMATION OF PLATELET-ERYTHROCYTE AGGREGATES IN PATIENTS ON HEMODIALYSIS

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Activated platelets may engage in dynamic interplay with other blood cells. We examined the evidence for platelet activation and the formation of platelet-erythrocyte aggregates (PEA) in chronic patients on hemodialysis (1). Whole blood obtained before and after 15 min of hemodialysis with both cuprophan and PMMA membranes was collected in vacum tubes and immediately fixed in 1.25% glutaraldehyde in 0.1M sodium phosphate buffer. For immunolocalization blood samples was embedded in Lowicryl K4M. Section were incubated with anti-fibronectin, thrombospondin, tenascin, and type IV collagen antibodies and with gold conjugated secondary antibody. Circulating activated platelets were higher than normal controls and further increased during hemodialysis sessions, the increase being higher when patients were dialyzed with cellulosic than with synthetic membranes. The formation of PEA was demonstrated by transmission electron microscopy, showing that platelets adhering to RBC had the typical morphologic signs of activation and presence of fibrillar material at platelet-erythrocyte point of contact. Immunolocalization showed no label for either tenascin or type IV collagen but a faint labelling was seen for thrombospondin in intraplatelet granules and on the erythrocyte surface. Fibronectin was observed in granules within platelets before dialysis whereas at 15 min of cuprophan dialysis it was detected with more intense immunoreactivity in granules close to cell membrane. We conclude that PEA occur in hemodialysis patients probably owing to a primary platelet activation mechanism.

1) Sirolli V. et al Thromb Haemost 2001,86, 834-9

IMMUNOLOCALIZATION OF THE RECEPTORS FOR UROKINASE PLASMINOGEN ACTIVATOR (uPAR) IN BLADDER CARCINOMA

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The urokinase plasminogen activator (uPA) acts mainly by activating plasminogen to proteolitically active plasmin when bound to its high–affinity receptor (uPAR): however in recent years growing evidence demonstrated that uPA and especially uPAR are also involved in other processes independent of plasmin formation such as chemotaxis and cell adhesion. uPAR expression has been reported in many human cancers where it has been correlated with tumor aggressiveness. As far as bladder cancer is concerned, very few information is available. Therefore we investigated, by means of quali-quantitative immunoelectron microscopy, the intracellular distribution of uPAR in normal and neoplastic (grade I, II and III urothelial papillary carcinomas) human bladder. Sections were processed for immunocytochemistry using the mouse anti-uPAR antibody R2 revealed by a specific gold-conjugated secondary probe. Electron micrographs were used for the quantitative evaluation of immunolabelling, expressed as number of gold grains/μm^2 of cytoplasmic and nuclear area and as number of gold grains/μm of cell surface membrane. Our results demonstrate a similar uPAR distribution in all samples: in the cytoplasm the signal was ubiquitously distributed, while in the nucleus the gold grains were preferentially located on the perichromatin fibrils and on the nucleolar dense fibrillar component. Quantitative evaluation revealed similar labelling densities in the cytoplasm and in the cell surface of all experimental groups, whereas in the nucleus of grade I carcinoma cells the signal was significantly higher than in the other samples.

APOPTOTIC CELL SURFACE: ASPECTS AND MECHANISMS

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Apoptosis is a fundamental process of cell death involved in the maintenance of homeostasis in multicellular organisms (Jacobson et al., Cell, 1997; Thompson et al., Science, 1995). Peculiar biochemical pathways characterize apoptotic death, followed by chromatin margination and formation of micronuclei, scattered throughout te cytoplasm. Moreover, cell shape is deeply affected and loss of adherence, changes in cell surface blebbing and fragmentation into apoptotic bodies appear (Atencia et al., Vitamins and Hormones, 2000). Here we describe the ultrastructural features of cell surface in HL-60, SK-N-MC and FO-1 cell lines treated with different apoptotic triggers. Cell morphology was investigated by transmission (TEM) and scanning electron microscopy (SEM) (Falcieri et al., Histochem. Cell Biol., 2000). AnnexinV-FITC labeling (Renò et al., Histochem. Cell Biol., 1998) and supravital PI staining (Zamai et al., Cytometry, 1996) were utilized to study biochemical membrane changes. DNA electrophoresis was performed to assess DNA fragmentation, as a hallmark of apoptosis. All lineages show apoptotic patterns in TEM, generally in the presence of a good preservation of membrane morphology. Nevertheless, SEM suggests its involvement, with microvilli disappearance and bleb formation. Furthermore, the adherent cell lines (SK-N-MC and FO-1) loose cell-cell and cell-substrate contact and show a suspended population of roundish cells, mostly evidentiating apoptotic features. Therefore, not only nucleus, but also cell membrane, can be considered an important target in apoptosis.

INVESTIGATION OF HG^{2+}-DEPENDENT CELL SIGNALLING IN TROUT HEPATOMA (RTH-149) CELLS BY MEANS OF CONFOCAL IMAGING

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Here we report different signalling events triggered by Hg^{2+}, on the RTH-149 trout hepatoma cells. Confocal imaging of fluo 3-loaded cells showed that Hg^{2+} is able to induce $[Ca^{2+}]_i$ changes and Ca^{2+} waves. The $[Ca^{2+}]_i$ rise was decreased by verapamil, a well known Ca^{2+} channel blocker, and abolished by addition of glutathione (GSH) in the extracellular medium. Conversely, the $[Ca^{2+}]_i$ transient was almost unaffected by cell loading with the heavy metal chelator TPEN or with esterified GSH. These data indicate an important effect of the metal at plasma membrane level. In Ca^{2+}-free medium, Hg^{2+} induced a lower $[Ca^{2+}]_i$ transient, that was abolished by manoalide, a Phospho-Lipase C inhibitor, or by cell loading with Guanosine-5'-O-(2-thiodiphosphate) (GDP-βS), a G protein inhibitor, or heparin, a blocker of InsP3 receptors. Also, cells loaded with heparin and exposed to Hg^{2+} in the presence of external Ca^{2+} showed a drastic reduction of $[Ca^{2+}]_i$ rise. Data indicate that Hg^{2+} induces both extracellular Ca^{2+} entry and InsP3-dependent intracellular Ca^{2+} release. These two processes seem to be interdependent, as Ca^{2+} release is amplified by Ca^{2+} entry through Ca^{2+}-induced Ca^{2+} release.

CYTOLOGICAL AND ULTRASTRUCTURAL ASPECTS OF MURINE MIELOMA CELLS TREATED WITH ALOE ARBORESCENS LEAF EXTRACTS

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Anti-inflammatory, wound-healing and immuno-stimulant properties of Aloe have been known since long time and a cytotoxic activity of those plants has been recently evidenced. Our previous studies showed the inhibition of P3X murine mieloma cells growth by alcoholic extracts from *Aloe arborescens* leaves (Rondini et al., Acta Phytotherapeutica, 2000). In the present study, by scanning and transmission electron microscopy, we investigated the cytology and ultrastructure of P3X cells treated with *Aloe arborescens* leaf extracts. An immunofluorescence study was also carried out by confocal microscopy using an antibody against alpha tubulin. Morphological alterations, such as an increasing in diameter and modifications of the cell surface were observed in treated cells using SEM. In particular the filamentous structures, normally slightly present on the surface of control cells, increased in number and dimension. Contemporaneously, TEM observation confirmed ultrastructural abnormalities in the cytoplasm of those cells, and evidenced numerous membranous inclusions thus suggesting degenerative processes. Confocal microscopy showed some modifications induced by leaf extracts such as the disappearance of the microtubule organization centre, and the depolimerization of microtubules or the alteration of their spatial distribution. Current studies are carrying out to identify the molecules involved in this cytotoxic activity.

NADPH-DEPENDENT REACTIONS CORRELATING WITH G6PD IN THE RAT OLFACTORY BULB

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The glucose-6-phosphate dehydrogenase (G6PD) is the first and rate-limiting step of the hexose monophosphate shunt, which provides pentose phosphates for nucleic acid synthesis and NADPH for reductive biosynthesis and detoxification reactions. Previous studies demonstrated that G6PD activity in the olfactory bulb (OB) was much higher than in other brain areas. Moreover, histochemistry and immunohistochemistry showed that the highest G6PD activity was localized in the olfactory nerve layer (ONL) and in the glomerular layer (GL). In this work, we investigated by means of histochemical and immunohistochemical techniques, the possible correlation between the G6PD activity and other NADPH-dependent enzymes: NADPH cytochrome P450 reductase (NADPH-P450R), glutathione reductase (GR) and NADPH-diaphorase (NADPH-d). The NADPH-P450R was prevalently localized in the ONL and in the GL. The GR was present in the ONL and in the GL, where it was mainly localized in periglomerular cells. Tufted cells showed a high GR concentration also. The NADPH-d showed a very high enzyme activity in zones of the ONL and in groups of glomerula, including some periglomerular cells. Granule cells had a marked NADPH-d activity also. The demonstration in the ONL and GL of high concentrations of G6PD, NADP-P450R, GR and NADPH-d accounts for a functional integration system among these enzymes and suggests that G6PD is the main source of NADPH in cited structures of the OB.

IONOTROPIC AND METABOTROPIC GLUTAMATE RECEPTORS IN THE RAT CEREBELLUM AFTER CISPLATIN INJURY

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Glutamate receptors mediate most of the excitatory neurotransmission in the mammalian central nervous system; NMDAR1, GluR2, GluR2/3 e mGluR1 are respectively three ionotropic and a metabotropic receptors that are expressed in the neural differentiation and development. The aim of this research was, at first, to demonstrate their role in the Purkinje cells differentiation and, then, to analyse both morphological and neurochemical patterns of Purkinje cells dendrite development in the damaged and reorganized cerebellar cortex, after cisplatin treatment at PD 10 (Pisu et al., Proceeding of FENS Forum, 2002). At this stage, controls showed immunoreactivity for NMDAR1 in the cytoplasm of Purkinje cells in all the cerebellar vermis lobules; soma and dendrites of these neurons were also labelled for GluR2, GluR2/3 and mGluR1. At early stage after cisplatin treatment, in the lobules VI-VIII, the reactivity for NMDAR1 was lower in several Purkinje cells, that in the outer zone of lobules appeared also small in size and irregularly shaped; moreover, GluR2 and GluR2/3 expression showed, in the molecular layer, a less extended dendritic trees of Purkinje cells and mGluR1 revealed weakly labelled or unlabeled areas. At the late stage (20 days) after the treatment, in the neocerebellar lobules (VI-VIII), mild granule cell ectopia, reorientation of some dendrite branches of Purkinje cells, and rearranged synaptogenesis patterns between Purkinje cells and interneurons were found. Our research evidences the differential morphological and chemical development of cerebellar lobules and, after damage, features of the late reorganization, that represents a strategy to promote the recovery of functionality after damages.

SURFACE LABELLING OF APOPTOTIC BODIES: A METHOD TO STUDY PHAGOCYTOSIS

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We have used N9 cells to follow, after phagocytosis, the fate of antigens present on the cell surface of apoptotic bodies, by morphological, cytochemical and immunocytochemical analysis at light/electron microscopy. Apoptotic thymocytes, labelled with the membrane-binding fluorochrome PKH2, are internalized by N9 cells and are visible as fluorescent spots inside the cytoplasm. Afterwards, the fluorescence spreads and finally is present as a diffuse labelling due to degradation of the internalized apoptotic thymocytes. We have also followed the fate of nuclear RNPs of apoptotic thymocytes during phagocytosis. In non-apoptotic cells, these RNPs occupy specific nuclear domains where transcription and splicing take place. During apoptosis, RNPs move towards the interchromatin space to form fibrogranular and heterogeneus complexes (HERDS) first extruded in the cytoplasm and, finally, abandoned within apoptotic bodies. These complexes can be followed by labelling the apoptotic bodies with an anti-Sm antibody. The antigen can be localized both at light and electron microscopy even after 2 h post phagocytosis, thus underlining the resistance to proteases of some RNP complexes.

MOUSE OVARIAN FOLLICLES CELLS SURFACES CONSTITUTING A MICROCANALICULAR COMPLEX: ULTRASTRUCTURAL IMAGING

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Aim. The Theca interna is the inner layer of large ovarian follicles, that produces androgens to be then converted in estrogens after their transport into the granulosa compartment. We analyzed by correlated scanning and transmission electron microscopy (SEM/TEM) the fine relationships between the theca cell (TC) surfaces in mouse developing and atretic follicles. *Materials and Methods.* Thirty mature mice were used. High resolution SEM procedures, without sputter coating, were performed on sonicated samples obtained with the employment of sonic frequencies from 5 to 15 KHz, modulated by 200 Hz, in a particular sonicator device. In this way, we were able to generate a gentle wave energy, which effected an optimal removal of extracellular matrix or cellular debris, with the consequent exposure of a well preserved epithelial surface. In addition, TEM methods using chemical tracers as ruthenium red and lanthanum nitrate, were employed. *Results and Discussion.* SEM/TEM images show a very tortuous and large system of intercellular lacunar spaces between TC, which open into interstitial spaces or associate to pericapillary spaces. Blebs and microvilli of TC are projected in these lacunae. Atretic follicles had a thecal capillary network with larger lumens and tortuosity versus normal growing follicles. TEM in association with chemical tracers allowed us to observe a preferential pathway existing towards interstitial spaces and the granulosa compartment rather than within thecal vessels. *Conclusions.* The highly integrated techniques TEM, SEM, sonication and use of chemical tracers, allow us to hypothesize that the functional meaning of this microlabirinthyc system around the cells and in the perivascular spaces represents a communicating system between granulosa and theca compartments.

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ABNORMAL MORPHOLOGY OF BOVINE SPERMATOZOA: SPERM HEAD DEFECTS IN A MARCHIGIANA BEEF BULL

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Introduction. There are several types of bovine sperm defects, such as spermatogenic abnormalities, which could lead to infertility; unfortunately, they are not always identifiable by conventional semen analysis techniques. Inversely, by means of TEM observations, we can evaluate more accurately all spermatozoa morphological features. *Objective.* The objective of the present study was to investigate the ultrastructural morphology of a Marchigiana beef bull sperm cells, which was subjected to a breeding soundness examination. *Methods.* An ejaculate was obtained by artificial vagina method and was subjected to routinely semen analysis. Semen aliquots were also routinely processed for TEM and examined on a Philips CM 12 STEM. *Results.* All semen parameters, such as volume of ejaculate, sperm concentration and motility, were within normal range of variability. Whereas, by TEM observations, several spermatozoa were characterized by macrocephalic or giant-headed sperm,

rolled head and nuclear crest, with its two edges curved resulting into a rounded profile and cell membrane appears to trapped membranous materials inside the curvature of the nucleus. In cross-section, sperm cells were crested with their two edges appearing forked. *Conclusions.* To our knowledge, this is the first report of the rolled head and nuclear crest in spermatozoa of Marchigiana breed; but further studies are needed to evaluate the effective impact of these abnormalities on sperm fertilizing ability.

PECULIAR ULTRASTRUCTURE OF SPERMATOZOA OF URODASYS ANOREKTOXYS (GASTROTRICHA, MACRODASYIDA)

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An ultrastructural study of the spermatozoon of *Urodasys anorektoxys* Todaro, Bernhard and Hummon 2000 was carried out. This is a new species found at about 500 m depth in the Santa Barbara Basin (California) in severely dysoxic water with a high sulphide content. TEM observations showed a filiform cell with spring-shaped portions in agreement with the sperm model described for the macrodasyids. However, it shows several peculiarities: the spiralized acrosome contains an axial tubular structure, the posterior part of which is wrapped by the nucleus; the nuclear chromatin is uncompletely condensed and organised into two distinct regions; mitochondria are absent; the 9x2+2 axoneme is enclosed by a thick sheath made of large spheres: it has the same position of the striated cylinder and the accessory fibers present in numerous macrodasyids. The absence of mitochondria is such an intriguing character that we also decided to study the spermiogenesis. Many mitochondria in the first and in the second spermatocyte are present, while from the initial spermatids they undergo a progressive transformation and reduction in size until they disappear in the final spermatids. The spermatozoon of *U. anorektoxys* is close to the model of macrodasyids in the acrosome structure, while both the nucleus and the tail appear to be autapomorphic, the first for the absence of mitochondria and the second for the presence of peculiar large spheres.

STATIC MAGNETIC FIELD INDUCES MORPHOLOGICAL AND BIOCHEMICAL MODIFICATIONS ON *FUSARIUM CULMORUM* MYCELIA GROWTH

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Fusarium are cereal pathogens causing diseases (e.g. Fusarium head blight in wheat and barley, Fusarium ear rot in maize) and also producing important mycotoxins of concern in animal and human health. In the present study we propose static magnetic fields (sMF) as physical anti-fungal agent after having examined the effect of 3,000±300 Gauss sMF on mycelia growth of *F. culmorum*. The results of our experiments reveal that growth inhibition of *F. culmorum* as a response to sMF exposure is accompanied by either morphological and biochemical changes. Scanning (SEM) and transmission (TEM) electron microscopy show shrivelled hyphae and increased vacuolisation similar to those occurring in plant pathogenic fungi treated with chemical fungicides observed by others [Kang et al., Pest. Manag. Sci., 57, 491, 2001]. Since it is already demonstrated that fungal virulence depends on the glyoxylate cycle activity, we show that the exposure of *F. culmorum* mycelia to sMF induces significant decrease of both isocytrate lyase (ICL) activity and glucose concentration. Furthermore the increased number of lipid bodies may correlate with the augmented total lipid concentration and lipase activity we have observed, by suggesting a synthetic rather than a hydrolytic action responsible for lipid bodies accumulation. We indicate the use of sMF to reduce the incidence of infection and virulence of plant pathogenic fungi.

MORPHOLOGICAL AND OUTER MEMBRANE PROTEIN PATTERN CHANGES IN H. PYLORI DURING CONVERSION FROM BACILLARY TO COCCOID FORM

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Conversion from bacillary to a fully coccoid form via an intermediate U- and V-shaped form has been described in prolonged cultures of *H. pylori*. This morphological transformation may be the expression of transitory adaptation to a particular environment and may play an important role in recrudescence or relapse of *H. pylori* infections. The aim of this study was to evaluate morphological and outer membrane protein changes in *H. pylori* during ageing-induced conversion to coccoid morphology.

We used two *H. pylori* strains (the reference NCTC 11639 and a fresh clinical isolate) cultivated in microaerophilic environment at 37 °C and monitored their morphological and biochemical evolutions every two days for 11 days. Microscopic examination revealed the passage from spiral to U- and V-shaped morphology after 5-8 days of incubation; conversion to coccoid form was observed between day 9 and 11. As to protein pattern, SDS-PAGE analysis showed no significant differences until day 5, while we detected an increase in the intensity of electrophoretic bands between 97,6 and 66,2 Kda from day 7 to day 9. Biochemical tests demonstrated not only a modification of outer membrane protein profiles, but also an intra-specific variability as resulted by comparison between the two strains analysed. Our findings suggest that structural and cell wall changes associated with coccoid transformation represent a typical response in *H. pylori* and may constitute a survival strategy in adverse environmental conditions.

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