18.12.2008 ore 12:25 – sessione II

Different effects of oncogenic H- and K-Ras expression on HT-29 colorectal carcinoma cell line.

Maurizio Bellavia, Fabrizio Miranda, Anna Maria Marotta, Mario La Farina, Ida Albanese

The Ras oncogene is mutated in about 30% of the human tumors and its mutations are almost always point mutations concerning codon 12, 13 and 61. These mutations cause in the proteins a reduced GTPase activity, so that they become costitutively active. In human cells there are threee main isoforms of Ras (H, K, N-Ras) which can trigger alternative pathways of signal transduction. In order to investigate the effects of expression of different oncogenic Ras isoforms in colorectal carcinoma cells (HT-29), we obtained stable clones of HT-29 cells transfected with cDNAs codifying H-RasG12V and K-RasG12V called respectively H12 and K12 and K-RasG13D called K13, under the control of an hormone-inducible promoter. We had previously observed changes in shape and growth rate when mutated Ras was expressed. We then analyzed the differential expression of genes such as the cell-cycle inhibitor p21 and the EMT associated gene snail. Q-RT PCR assay showed an increase in p21 level only in H12 cells but not in K13 and K12 compared with the respective controls. Instead, snail mRNA level was increased in all three clones. We investigated also by Semiquantitative RT-PCR H-RasG12V, p21 and snail levels in H12 cells induced for a long time and we noted a decreased level of H-RASG12V associated with no p21 and snail expression. Our results show that specific mutations of different Ras isoforms have different effects on morphology and gene expression in HT-29. Other studies are of course necessary to shed more light on the role played by Ras mutations in tumor devlopment.

18.12.2008 ore 12:50 – sessione II

Strategies in experimental models for evaluating apoptosis

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Apoptosis is a process that eliminates superfluous or genetically compromised cells. Cell death is one of the most important aspects of organization of the developing, as alteration in timing, level, or pattern of cell death can lead to developmental anomalies. In our studies we increased knowledge of the pathways of cell death involved in sea urchin *P.lividus* embryo development and we identified caspases activated by specific external stimuli. Our training was used as pilot study for evaluating apoptosis in follicular somatic and germ cells of patients with sterility problems. We found that cell death occur in human germinal vescicle, in a part of mature oocytes and in the oocytes that failed fertilization after ICSI (intracytoplasmic sperm injection), this failure was strictly correlated with apoptosis. Later, our interest was to investigate normogonadotropic women, undergoing to induction of multiple follicular growth by r-FSH, who showed an insufficient ovarian response in terms of follicular growth (low responders). We investigated the effects of r-LH supplementation in these low responders patients, valuating the apoptosis rate in cumulus cells: r-LH improved the chromatin integrity of these cells that can use as oocyte quality indicator. In addition, hypogonadotrope hypogonadism patients, undergoing *in vitro* fertilization programs, treated by r-FSH supplementation we found an improve of sperm parameters and apoptosis rate. Further study will be necessary to understand as improve integrity of DNA of somatic and germ human cells and increase IVF (in vitro fertilization) outcome.

18.12.2008 ore 15:00 – sessione IV

Ligand/receptor interactions that regulate gastrointestinal smooth muscle contractility

Serio Rosa, Zizzo Maria Grazia, Mulè Flavia , Amato Antonella, Baldassano Sara

One way for cells to communicate with their environment is by releasing substances, which interact with a class of proteins known as receptors, channels or intracellular targets to activate or deactivate other cells. Many different signalling molecules have been identified, including peptides (tachykinins, VIP, GLPs), purines (ATP and adenosine), amino acids (GABA) or gaseous substances (NO). Cellular communication is the way to maintain organism's stability and homeostasis and to regulate organ functions, e.g. gastrointestinal (GI) motility.
GI tract possesses a multitude of specific binding proteins, located mainly on smooth muscle cells and neuronal structures in the enteric nervous system, coupled to intracellular mechanisms that mediate changes in the muscular contractile state.
We used in vitro technical approaches (organ bath, intracellular microelectrode recording) to determinate the role of some chemical substances in the regulation of GI muscle function.
Signalling molecules interacting with membranal or intracellular receptors, *via* effector enzymes, decrease or increase the concentration of the intracellular second messengers (cAMP, cGMP or Ca^{2+}), that in turn modulate the opening of ionic channels.