1028 ORAL

Activation of stat3 transcription factor is involved in Rhoa GTPase oncogenic transformation

A. Salvador[1], S. Aznar Benitah[1], P.F. Valeron[2], S. Victoria del Rincon[1], L. Fernandez Perez[2], R. Perona[1], J.C. Lacal[1]. [1] *Biomedical Research Institute, Molecular and Cellular Oncology Department, Madrid, Spain;* [2] *University of Las Palmas, Molecular Biology Department, Las Palmas, Spain*

Purpose: RhoGTPases are members of the Ras superfamily involved in critical cellular funtions such as cell growth, development, apoptosis, cell cytoarchitecture and cell adhesion, among others. As well, they have been linked to both tumorigenic and metastasic processes. We have investigated the modulation of transcription factor Signal Transducer and Activator of Transcription 3 (Stat 3) activity in oncogenic Rho-transformed cells, and its role in Rho transformation. Methods: Ectopic expression in Human Embryonic Kidney (Hek293) cells, Western Immunoblot analysis against both tyrosine and serine phosphorylated endogenous Stat3, as well as DNA binging activity by Electrophoretic Mobility Shift Assays (EMSA) were performed to assess Stat3 activation by RhoA. Transcriptional activity of Stat3 was determined using reporter assays by subcloning a Stat3-Inducible DNA Element (SIE) upstream of a CAT gene. As well, kinase assays, chemical inhibition and expression of dominant active/negative mutants of different serine and tyrosine kinases were carried out. Anchorage-independent growth in soft agar of transfected cells was performed to determine oncogenic potential of transfected cells. Results: We have found a novel signaling pathway, whereby oncogenic RhoA efficiently induces both tyrosine and serine phosphorylation of Stat3, both necessary for its full transcriptional activity. Tyrosine phosphorylation of Stat3 by RhoA is necessary for its DNA-binding activity, and is exerted by both Janus Kinase (JAK-2) and Src family member Lck. RhoA-induced serine phosphorylation of Stat3 is exerted by Jun N-Teminal Kinase (JNK-1), but not Extracellular Regulated Kinases (ERK-1/2) or p38 MAPKs, and is essential for its full transcriptional activity. Furthermore, co-expression of wild-type non-transforming Stat3 with RhoA significantly enhanced the oncogenic potential of the latter. Accordingly, two different dominant negative mutants Stat3 proteins abrogate RhoA-induced transformation. Conclusions: Stat3 might be an important player in RhoA mediated tumorigenesis. Given that RhoA is overexpressed in a variety of human carcinomas (breast, colon, pancreas and kidney), and that Stat3 activity is greatly enhanced in a high percentage of several human tumors, a functional link of both these proteins may constitute a plausible pathway for the development of drugs with antineoplastic activity.

1029 ORAL

Physical interaction with yes-associated protein enhances p73 transcriptional activity

S. Strano[1], O. Monti[1], A. Baccarini[1], M. Sudol[2], A. Sacchi[1], G. Blandino[1]. [1] *Regina Elena Cancer Institute, Experimental Oncology-Molecular Oncogenesis Lab, Rome, Italy;* [2] *Mount Sinai School Of Medicine, Medicine, New York, USA*

Specific protein-protein interactions are involved in a large number of cellular processes and are mainly mediated by structurally and functionally defined domains. Here we report that the nuclear phosphoprotein p73 can engage in a physical association with the Yes-associated protein (YAP). This association occurs under physiological conditions as shown by reciprocal co-immunoprecipitation of complexes from lysates of P19 cells. The WW domain of YAP and the PPPPY motif of p73 are directly involved in the association. Furthermore, as required for ligands to group I WW domains, the terminal tyrosine (Y) of the PPPPY motif of p73 was shown to be essential for the association with YAP. Unlike p73a, b, and p63a, which bind to YAP, the endogenous as well as exogenously expressed wild-type p53 (wt-p53) and the p73g isoform do not interact with YAP. Indeed, we documented that YAP interacts only with those members of the p53 family, which have well conserved PPxY motif, a target sequence for WW domains. Overexpression of YAP causes an increase of p73a transcriptional activity. Differential interaction of YAP with members of the p53 family may provide a molecular explanation for their functional divergence in signaling.

1030 ORAL

Promotion of microtubule assembly in vitro by novel 28-KDA protein purified from human placenta

B.D. Hwang[1,3], Y.C. Lee[1], D.S. Huh[1], J.I. Park[1], S.T. Kwak[4], W.H. Yoon[2,3], S.K. Park[1,3], K. Lim[1,3]. [1] *College of Medicine, Chungnam National University, Department of Biochemistry, Daejeon, Korea;* [2] *College of Medicine, Chungnam National University, Department of Surgery;* [3] *Cancer Research Institute;* [4] *Kunyang University, Chungnam, Korea*

Purpose: Taxol-like protein-35 (TALP-35) was purified and characterized from human term placenta and the TALP-35 has antimetastatic and antiangiogentic effect. To identify another taxol-like protein, we have purified and characterized novel 28 kDa protein to promote microtubule assembly in vitro from human term placenta.

Methods: Microtubule proteins were prepared from bovine brain by a modification of the method of Hamel and Asnes. Tubulin polymerization was monitored by turbidimetry and electron microscopy. The change in absorbance at 350 nm was measured with a Gilford Model 2600 spectrophotometer with a thermostat cuvette attachment. Cosedimentation assay was performed to assess whether the TALP-28 directly bound to tubulin. Polyclonal antibody against to TALP-28 was developed and the locatization of TALP-28 was identified by immunostain.

Results: Novel microtubule assembly promoting-protein (taxol-like protein, TALP) was purified from human term placenta by combination of high salt extraction, phosphocellulose and 2 cycle hydroxyapatite column chromatography. Molecular weight of purified protein was identified as 28 kDa on SDS-PAGE. In vitro, TALP-28 increased the rate and extent of microtubule assembly in dose-dependent manner and its activity was greater 20 times than taxol when compared on molar concentration basis. In cosedimentation assay, polymerization of the tubulin was increased as a function of TALP-28 concentration, and TALP-28 was incorporated with the microtubule pellet fractions. TALP-28-induced tubulin polymer were morphologically normal microtubules. TALP-28 also induced microtubule assembly in the presence of 4 mM Ca2+, 10 mM colchicine, 10 mM vincristine, 240 mM NaCl, and low temperature (10oC). Microtubules formed with TALP-28 were resistant to cold temperature, 4 mM CaCl2, 240 mM NaCl, 5 mM GDP and 50 mM podophyllotoxin. TALP-28 was detected in the cytosol of placental trophoblast cell by immunostaining used of anti-TALP-28 polyclonal antibody.

Conclusion: TALP-28 binds to the tubulin, and then induces microtubule assembly and stabilizes microtubule. The action of TALP-28 on the microtubule polymerization is similar to taxol but the effect is stronger than taxol

[This study was supported by a grant of the Korea health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (HMP-00-B-20900-0900)].

1031 ORAL

Direct determination of intracellular oxygen concentration in vitro: are cultured tumour cells hypoxic?

T. Seppi, B. Forthuber, P. Lukas. *University of Innsbruck, Dept. of Radiotherapy - Radiooncology, Innsbruck, Austria*

Purpose: Tissue cultures (usually batch cultures), widely used to study molecular phenomena related to tumour ischemia/hypoxia or to drug resistance and radiosensitivity, are generally thought to be relatively well oxygenated in combination with an optimised nutrient supply when maintained under standard culture conditions (95% air, 5% CO2). However, due to technical limitations of existing oxygen measurement devices, only little reliable data is available regarding the intracellular oxygen concentration (in-vivo as well as in-vitro) which is thought to be crucial for enhanced radiosensitivity. Even if a great variety of data exists concerning the interstitial or pericellular oxygen concentration of tumours or cultured cells, these data do not necessarily reflect the intracellular situation. Therefore, it would be of great interest to monitor the intracellular oxygen concentration depending on changing metabolic environmental situations.

Methods: Different monolayers of tumour cell lines and primary cultures of normal cells are exposed to varying culture conditions. The intracellular oxygen concentration is continuously monitored by a patented LC-ECD-based measurement device with nanomolar resolution (limit of detection: 0.2×10^{-9} M O2), which has recently been developed and described by our research group. The expression of metabolic key enzymes and the concentration of metabolites in the supernatant culture medium is correlated to the given intracellular oxygen concentration during long-term culture.

Results: Cell lines differ extremely regarding their intracellular oxygenation status depending on cell type, changing nutrient supply and the chosen culture method. In some metabolically fully active and normally dividing cultured tumour cells, oxygen concentrations are reached which are far below the limit of detection of commercially available oxygen microprobes i.e.