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

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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.

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Physical interaction with yes-associated protein enhances p73 transcriptional activity

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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.

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

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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

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

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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.

down to 50 nM O2 corresponding to 0.025% of the saturated concentration prevalent on the surface of the overlaying medium (196 μM O2).

Conclusion: These data strongly suggest that severe hypoxia (in terms of very low intracellular oxygen concentrations) is a normal situation for some tumour cells cultivated under standard conditions due to their elevated nutrient and oxygen consumption rates. On the other hand, a strong oxygen gradient built up by mitochondrial respiration increases the oxygen flux via enhanced diffusion rates thereby continuously providing oxygen.

Supportive care & quality of life

1032 ORAL

Improving the quality of pain treatment by a tailored Pain Education Program for cancer patients in chronic pain

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It has become increasingly evident that patients' knowledge and attitudes regarding pain is important for cancer pain relief. Educational interventions can affect pain treatment. However, the lack of well-established outcome measures to evaluate adequacy of pain treatment hampers the evaluation of educational pain programs

In this study, the effectiveness of a Pain Education Program (PEP) in cancer patients with chronic pain was investigated in a randomized controlled clinical trial. The main purpose of this study was: 1) to assess the adequacy of pain treatment; and 2) to evaluate the effects of the PEP. The PEP was tailored to the needs of the individual patient and consisted of three elements: 1) educating patients about the basic principles regarding pain and pain management; 2) instructing patients how to report their pain in a pain diary; and 3) instructing patients how to communicate about pain and how to contact health care providers. Intervention group patients received the PEP in the hospital and postdischarge by nurses who were specially trained as pain counselors. Follow-up assessments were till 8 weeks postdischarge.

A total of 313 pain patients were studied. Adequacy of pain treatment was evaluated by means of the Amsterdam Pain Management Index, a measure that compares the aggregated scores of patients' Present Pain Intensity, Average Pain Intensity, and Worst Pain Intensity, corrected for patients' Tolerable Present Pain, with the analgesics used by the patient.

In the hospital, 59.8% received less than optimal analgesic treatment. Results showed that the PEP proved to be feasible, showed a significant increase in pain knowledge in patients who received the PEP, and a significant decrease in pain intensity. Postdischarge, the intervention group patients were significantly more adequately treated than the control group patients. These findings suggest that quality of pain treatment in cancer patients with chronic pain can be enhanced by educating patients about pain and improving active participation in their own pain treatment. The benefit from the PEP, however, decreases slightly over time, pointing at a need for ongoing education.

1033 ORAL

Patients treated with an NK1 receptor antagonist report less hardship due to chemotherapy-induced nausea & vomiting compared to those on standard antiemetic therapy

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While the antiemetic properties of NK-1 receptor antagonists have been reported in the literature, little information exists on the positive impact of these compounds on patient-reported outcomes. Specifically, the ability to avoid personal hardship and hardship on others due to nausea and vomiting is likely to be an important benefit to patients. In an international randomized double-blind Phase IIb trial, 228 cisplatin-naive patients (47% female, mean age 55) treated with cisplatin * 70mg/m2 received either triple antiemetic therapy (MK-0869 125mg+dexamethasone (D) 20mg+ondansetron (O) 32mg on day 1 followed by MK-0869 80mg+D 8mg on days 2-5) or standard therapy (D 20mg + O 32mg on day 1 followed by D 8mg on days 2-5). Patients recorded vomiting episodes, nausea ratings and rescue medications in a daily diary. Five days post-chemotherapy, patients completed the Functional Living Index-Emesis (FLIE), a measure of the impact of nausea and vomiting on daily life. Cross-culturally validated translations of the diary and FLIE were used in all international sites. "No impact on daily life" (NIDL) is defined as a FLIE average item score >6 on a 7-point scale. The % of patients reporting NIDL from nausea and vomiting in each treatment group (MK-0869 vs standard therapy) was assessed by the FLIE total score (85** vs 67), Nausea domain score (76* vs 60), and Vomiting domain score (93** vs 68). Additionally, the % of patients in each treatment group reporting NIDL from nausea, specifically related to personal hardship (80* vs 64) and hardship on others (83 vs. 74), and the % reporting NIDL from vomiting, specifically related to personal hardship (93** vs 68) and hardship on others, (96** vs 71) was assessed. (*$p<0.05$, **$p<0.01$). Nearly 20% more patients treated with MK-0869 reported NIDL as assessed by the FLIE total score over the 5 days post-chemotherapy compared to those on standard antiemetic therapy. Likewise, significantly more patients treated with MK0869 reported no impact of nausea on daily life specifically related to personal hardship and no impact of vomiting on daily life specifically related to both personal hardship and hardship on others. Patients treated with MK-0869 are better able to maintain daily life and avoid hardship following highly emetogenic chemotherapy.

1034 ORAL

Cancer clinical trials within Europe – An examination into EORTC QL studies

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Purpose: For cancer patients, Quality of Life (QL) is now becoming an increasingly accepted endpoint in cancer clinical trials. However, reports in published literature suggest that fewer than 10% of all clinical trials have quality of life assessment, although it is believed such reports may be biased by time lag factors in the new and emerging field of QL. This paper therefore examines the extent of quality of life studies that are conducted within one of the largest cancer clinical trials organizations in Europe, the EORTC, investigating both completed and ongoing clinical trials.

Method: An examination of all clinical trials conducted by the EORTC (between January 1990 and January 2000) was undertaken, by reviewing data from various databases, publications and records. Trials were systematically selected if they involved any aspect of QL, as clearly specified in the protocol.

Results: In total, some 112 EORTC clinical trials were identified as having a QL sub-study. Over 10,000 patients had been entered into active trials. All of the trials involved multinational patient recruitment, with the highest recruitment from The Netherlands, France and Germany and the lowest from Malta, Estonia and Slovakia. Approximately 14 disease groups have been actively recruiting patients over the last decade, with the major number of patients being from disease groups of Genitourinary, Breast and Lung cancers. A clear linear trend was noted, with increasing numbers of clinical trials involving QL components over this ten-year period. Of all the trials, 74 studies were phase III design, 15 were phase II design, and the remainders were feasibility studies. Presently, 45 trials are ongoing, and open to patient entry, 19 are nearly mature for data analysis, 15 have now being published and 10 are now being analyzed. In the last year, 30 new studies have been submitted for research involving QL, suggesting quality of life is a highly important endpoint in present day trials across the European setting.

Conclusion: While QL was not a major component of EORTC clinical trials in the early 1990's, it is now highly integrated into EORTC cancer clinical trials, and almost a standard secondary endpoint. This suggests that clinicians and researchers alike in the European context are increasingly seeing the importance of patient based outcome assessment methods for assessing the value of cancer therapeutic modalities.

1035 ORAL

Neuroprotective effect of glutathione (GSH) on oxaliplatin (L-OHP)-based chemotherapy in advanced colorectal cancer patients (pts): a randomized double-blind placebo-controlled trial

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Purpose: L-OHP is a platinum compound active in colorectal cancer and