performing tumor tissue microarray analysis of clinical lung cancer materials.

Preliminary results: Gene expression microarray study: RNA from 28 primary NSCLC, 8 samples of normal lung that were taken from the same patients, seven independent brain metastases and one specimen of normal brain (commercial RNA that is derived from a pool of normal brains) were hybridized to Affymetrix U95 Chips (containing 12625 probe sets). Of the 28 primary NSCLC cases 6 developed brain metastases and 7 extra-cranial metastases during a minimal follow-up of three years. Limited space precludes a detailed description of the analysis). The microarray results were confirmed by RQ-PCR of selected genes. ADAM8 and N-cadherin are according to these analyse genes associated with brain metastasi in NSCLC patiens screned above. After werrification on symplex from independent NSCLC patient files, collected both in Israel and Czech republic we found a significant association between n-Cadherin expression and brain metastasis (p= 0,008).

Conclusion: n-Cadherin is a wery strong predictor of brain metastasis in NSCLC patiens.

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Pilot study of neo-adjuvant intra-arterial (i/a) chemotherapy in patients with sarcomas of a head and neck

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Purpose. Reduction the volume of operation in patients with sarcomas of head and neck.

Methods. 19 pts (6 female, 13 male) with various histological types of head and neck sarcomas, age 19-59 years (middle age 39 years). Primary sarcomas were diagnosed at 15 people, recurrent at 4. Sarcomas of soft-tissue (SST) G1-G2 were diagnosed for 6 pts, the primary bone sarcomas G1-G2 at 13. Treatment regimen were: doxorubicin 45 mg/m2 i/a for 1 hour 1-2 days, cisplatin 75 mg/m2 i/a for hour, 1-2 days with one-stage intravenous hydratation, cyclophosphamide 800 mg/m2 intravenously 3 day. Interval between cycles was 14 days; number of cycles were 2-4. For i/a chemotherapy was used a. carotis externe.

Results. Clinical efficacy was registered at 15 pts (SST 4, bone sarcomas 11). According to beam methods of diagnostic, partial response was noted at 10 pts, stabilization at 5. All of them were underwent operation. Follow-up period in this group was from 5 till 22 months. At 4 pts treatment was inefficient. After 4 month the progress of disease was noted at 3 people and local recurrence of tumor in the same time at 1. Medical pathomorphosis III was noted in 4 cases (including bone sarcomas G1-G2), accordingly, changes of II and I wers noted in 8 and 7 cases.

Conclusion. Preoperative intra-arterial chemotherapy allows to reduce the volume of surgery, in the patients with widespreaded sarcomas of head and neck.

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A functional link between the MRN complex and the Gcn2p kinase uncovered by the antitumour drug beta-lapachone

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Background: Beta-lapachone (b-lap) is an anticancer agent that selectively induces cell death in several human cancer cells. We previously reported that, in budding yeast, b-lap was cytotoxic, induced DNA damage and activated a G1/S Mre11-Tel1p checkpoint pathway preceding death. Our aim was to gain further insights into the mechanism of b-lap action and identify the molecular targets of b-lap action.

Materials and methods: We compared the gene expression profile of the b-lap treated yeast cells with that obtained from untreated cells using cDNA microarrays. We used Significance Analysis of Microarrays to identify differentially expressed genes between untreated/b-lap treated cells. The data obtained after analysis of the microarray was validated by standard yeast genetics and molecular biology approaches.

Results: Interestingly, numerous amino acid biosynthesis genes were found to be regulated by the drug, suggesting that b-lap might activate the General Control of Nutrients (GCN) pathway in yeast. Accordingly, b-lap treatment incremented phosphorylation of the eIF2 alpha subunit in a GCN1, GCN2 and GCN20-dependent manner. Suriprisingly, phsophorylation of eIF2alpha was fully dependent on the MRN complex. Furthermore, Gcn2p kinase modulated i) checkpoint responses triggered by b-lap treatment, and ii) cell viability in response to b-lap exposure. finally, we found that Gcn2p regulated checkpoint function by mechanisms other than eIF2α phosphorylation.

Conclusions: These data uncover a functional link between the Gcn2p kinase and the MRN complex and suggest that Gcn2p may have additional functions besides regulating translation.

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Sensitization of breast cancer cells to anthracyclines by docosahexaenoic acid through loss of glutathione peroxidase response

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We found long-chain n-3 polyunsaturated fatty acids, such as docosahexaenoic acid (DHA), to enhance the sensitivity of human breast cancer cell lines (Germain 1998) and rat mammary tumors to chemotherapy containing agents that induce an oxidative stress (Colas 2004), such as doxorubicin. Since DHA, with its 6 double-bonds, is very prone to oxidation, its membrane incorporation would provide more abundant targets for ROS generated by doxorubicin. To examine the relation between chemo-sensitization by DHA and tumour cells oxidative and antioxidant status, we used two breast cancer cell lines: MDA-MB-231, in which DHA increases sensitivity to doxorubicin, and MCF-7, which is not chemosensitizable by DHA. Upon anthracycline treatment, reactive oxygen species (ROS) and lipid peroxidation levels were enhanced only in MDA-MB-231 under DHA supplementation (30 μM). This was concomitant with a decrease of cytosolic glutathione peroxidase (GPx1) activity by 30%, a crucial enzyme for protection against hydrogen and lipid peroxides, and an accumulation of glutathione, the GPx co-substrate. This lack of GPx response resulted from a decreased amount of GPx protein.

We used an autochthonous rat mammary tumour model to investigate in vivo the DHA effect on GPx1 activity and on anthracyclines treatment efficacy. Rats were fed a control diet and a DHA-enriched diet (3.6 % of DHA in the diet). When the tumour reached 1.5 cm², rats received 1 injection of epirubicin (2.5 mg/kg via intraperitoneal route) per week during 6 weeks. We found that dietary DHA enhanced tumour sensitivity to epirubicin and this effect was associated to a decrease of GPx1 activity by 20%. Furthermore we found an inverse correlation (r^2=0.488) between epirubicin efficacy and GPx activity. Conversely, when antioxidant vitamin E was added, tumour GPx1 activity was restored and the DHA effect on chemosensitization was abolished.

Thus, loss of GPx response to an oxidative stress in tumour cells may account for the ability of peroxidizable targets such as DHA to enhance tumour sensitivity to ROS-generating anticancer drugs.

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The G-quadruplex ligand RHPS4 interferes with telomere replication leading to ATR-dependent DNA damage response

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Functional telomeres are required for the replicability of cancer cells. The G-rich strand of telomeric DNA can fold into a 4-stranded structure known as G-quadruplex (G4), whose stabilization by specific ligands, can limit telomere function and cancer cell growth. RHPS4 is a telomere-interactive molecule possessing antitumoral activity because of its ability to rapidly induce telomere dysfunction and cell death [1].

Here, we show that RHPS4 induces a potent DNA damage response specifically in S-phase cells. In particular, we show that in S-arrested cells treated by RHPS4 ATR, but not ATM, is required for the formation of phospho-H2AX foci colocalizing with proliferating cell nuclear antigen (PCNA), BRCA1 and 53BP1. Interestingly, ATM is phosporylated at Ser1981 but in contrast to ionizing radiations, this activation of ATM is strictly ATR dependent, suggesting that the cellular response to pharmacological telomere deprotection follows a pathway that, most likely, represents ATR activation in response to replicational stress. By combining BrdU incorporation with CHIPs assay we clearly demonstrated that RHPS4 interferes with the replication of the telomeres, altering the dynamic association of the telomeric proteins TRF1, TRF2 and POT1. Interestingly, RHPS4 does not induce a specific DNA damage at an interstitial telomeric sequence, suggesting that it interferes with a terminal event of telomere