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CROSSTALK BETWEEN P53 FAMILY AND HYPOXIA INDUCIBLE FACTOR IN CANCER. © *Ivano Amelio*, *Gerry Melino.* Medical Research Council, Toxicology Unit, Leicester, UK.

HIFs have long been associated with resistance to therapy, metastasis, and poor survival rates in cancer patients. In parallel, although the tumor-suppressor p53 acts as the first barrier against tumor transformation, its inactivation also appears to be crucial for enabling cancer progression at advanced stages. Crosstalk between HIFs and the p53 family might act as a determinant of cancer progression through regulating angiogenesis, the tumor microenvironment, dormancy, metastasis, and recurrence. Our data demonstrates that the tumor suppressor TAp73, a member of the p53 family of genes, opposes HIF-1 activation in cancer cells, resulting in reduced angiogenesis and tumor progression. TAp73-depleted mice show increased tumorigenicity, associated with increased HIF-1 signaling and angiogenesis. Expression of TAp73 in human cancers predicts good survival outcome and retrocorrelates with HIF-1 expression and activation. In addition, expression of mutant forms of p53 in cancer cells affects p73 antagonism on HIF-1 expression, thus promoting cancer progression. We suggest that a complex interfamily crosstalk p53 family/HIF plays a critical role in cancer pathogenesis.

THE REGULATION OF AUTOPHAGY IN MAMMALS: THE ROLE OF AMBRA1. © *Manuela Antonioli*,[1] *Gian Maria Fimia*,[2, 3] *Mauro Piacentini*.[1, 2] [1]Department of Biology, University of Rome «Tor Vergata», Rome, 00133, Italy, [2]National Institute for Infectious Diseases I. R. C. C. S. «Lazzaro Spallanzani», Rome, 00149, Italy, [3]Department of Biological and Environmental Sciences and Technologies (DiSTeBA), University of Salento, Lecce 73100, Italy.

Autophagy is a basic cellular phenomenon essential for maintaining cellular homeostasis by the lysosomal degradation of protein aggregates, damaged organelles, and recyclying their components. It is involved both in physiological and pathological conditions, including cancer. The role of autophagy in cancer is controversial due to its known participation both in tumor suppression and tumor survival. Thus the comprehension of the molecular mechanisms regulating this important intracellular event represents the basis for its application in cancer therapy. One of the key and not fully understood aspect of the autophagy response in mamalian cells is represented by its rapid and transient induction. We have recently demonstrated that the E3 ubiquitin ligases Cullin-5 and Cullin-4 act as key regulators of the induction and termination of autophagy by their dynamic interaction with AMBRA1, an essential element of the Beclin1 complex regulating autophagy. Under steady state physiological conditions, Cullin-4 binds to AMBRA1 leading to its degradation by the proteasome. By contrast, autophagy induction promotes AMBRA1 stabilization by its release from Cullin-4 mediated by ULK1. Interestinglly, the Cullin-4/AMBRA1 dissociation is transient, and the re-established interaction triggers AMBRA1 degradation, terminating the autophagy response. Furthermore, upon the Cullin-4 release, AMBRA1 interacts and inhibits Cullin-5, thus promoting the accumulation of the mTOR inhibitor DEPTOR that ensures the rapid onset of autophagy. These findings show that Cullin-mediated modulation of the levels of autophagy key regulators such as Ambra1 and DEPTOR dynamically controls the autophagy response. Considering that the dysregulation of cullin activity has been shown to contribute to oncogenesis our findings open a new avenue for their application in cancer therapy by the regulation of autophagy.

THE INHIBITOR OF GROWTH ING3 STIMULATES PROLIFERATION OF PROSTATE CANCER CELLS. © *Urszula McClurg*,[1] *Svitlana Korolchuk*,[2] *Byron Matthiopoulos*,[1] *Craig N. Robson*,[1] *Rémy Pedeux*,[2] *Olivier Binda*.[1] [1]Newcastle Cancer Centre at the Northern Institute for Cancer Research, Newcastle University, Paul O'Gorman Building, Medical School, Framlington Place, Newcastle upon Tyne, England, NE2 4HH,[2] INSERM U917, Microenvironnement et Cancer, Université de Rennes 1, Établissement Français du Sang Bretagne, Rennes, France, olivier.binda@newcastle.ac.uk

Despite their fundamental importance in cancer, the molecular mechanisms that regulate the access to genetic information remain incompletely defined. One recently identified mechanism is based on the association of proteins (readers) with the scaffolding histone proteins that condense the genome within the nucleus of the cell. These readers recruit enzymes to open or close the structure of the genome, thereby regulating access to genetic information. Aberrant access to genetic information leads to human pathologies, including cancer.

Members of the inhibitor of growth (ING1-5) family of histone mark readers associate with methylated histones to regulate chromatin signalling. As reported for other ING protein, ING3 regulates p53-dependent transcription. In additon, as a subunit of the TIP60 histone acetyltransferase complex and an essential component for effective TIP60 activity on nucleosomes, ING3 may also regulate androgen receptor AR-dependent transcription and play a role in prostate physiology.

Unexpectedly for a candidate tumour suppressor, we observed that silencing of ING3 expression by siRNA inhibits the proliferation of several human prostate cancer cell lines, independently of AR status. The reduced proliferation was characterized by increased G_1/S ration, sub-G_1 population, and apoptosis. Generally, ING family members function by bridging HDAC or HAT complexes to chromatin by associating with histone H3 trimethylated on lysine 4 ($H3K4^{me3}$) via the carboxy terminal plant homeodomain (PHD). Thus, to dissect the unexpected ING3-mediated growth stimulation, we have predicted the structure of the PHD domain of ING3 and determined that the conserved tyrosine 362 (Y362) and tryptophane 385 (W385) are involved in the association with $H3K4^{me3}$. Indeed, mutation of either Y362 or W385 greatly reduced the association of ING3 with methylated histones. To understand how ING3 regulates cellular proliferation, we conducted a gene expression analysis and uncovered a network of cell cycle genes that are regulated ING3. Specifically, silencing of ING3 expression led to decreased cellular proliferation marked by increased expression of genes including BAX, p21 (CDKN1A), and TMPRSS2, and reduced expression of AURKA, CCNA2, and CCNB2, while exogenous expression reduced their expression in a PHD-dependent fashion.

Together, our results demonstrate that ING3 expression in prostate cancer cell lines stimulates cellular proliferation by controlling the expression of cell cycle, androgen receptor-dependent, and p53-dependent genes.

NOVEL INTERACTIONS OF PIRH2 PROTEIN AND THEIR POTENTIAL SIGNIFICANCE IN CANCER. © *Alexandra Daks*,[1, 2] *Olga Fedorova*,[1, 2] *Alexey Petukhov*,[1, 2] *Oleg Shuvalov*,[1] *Elena Vasileva*,[1] *Nickolai Barlev*.[1, 2] [1]Institute of Cytology RAS, St. Petersburg, St. Petersburg, [2]St. Petersburg State Institute of Technology, St. Petersburg, Russia, alexandra.daks@gmail.com

Pirh2, the product of RCHY1 human gene, is one of the key ligases that promote p53 ubiquitination and degradation. While the role of the p53 protein in tumor formation is well established, the role and involvement of Pirh2 in carcinogenesis and malignant transformation is controversial. For example, the Pirh2 protein is overexpressed in about 84 % human lung cancers (1). High expression of Pirh2 is frequently detected in many clinical tumor tissues including hepatocellular carcinoma (HCC), prostate cancer, head and neck cancer and is associated with poor prognosis (2), (3), (4). On the other hand Pirh2 was shown to act as tumor suppressor via degrading c-Myc and low expression of human Pirh2 in lung, ovarian, and breast cancers correlates with decreased patients' survival (5). Thus, despite evidence of Pirh2 participation in tumorigenesis its specific role in this process remains unclear and requires further investigation.

We tried to decipher the role of Pirh2 in tumorigenesis by investigating its interacting protein partners in tumor cells, which may also be the targets of Pirh2-mediated ubiquitinylation. To this end, we incubated purified recombinant GST-Pirh2 protein with cell extracts followed by MALDI-TOF mass spectrometry. As a result we identified more than 100 proteins that have not previously been described to interact with Pirh2. We focused on those proteins which are known to participate in apoptosis or cancer formation and progression. Thus we have confirmed interaction of Pirh2 with such proteins as Ku70, Parp1, Elavl1 and Set7/9 by reciprocal GST-pulldown or co-immunoprecipitation methods. Furthermore, we also showed that Pirh2 mediated ubiquitinylation of several interacting proteins. Certainly, the functional significance of these interactions requires further investigation. However, this work underpins the research of Pirh2-associated cancer formation and progression mechanisms.

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MODULATION OR TUMORGENECITY BY HDJ2 MOLECULAR CHAPERONE. © *D. Meshalkina*, *I. Guzhova*, *M. Shevtsov*, *B. Margulis.* Institute of Cytology RAS, St. Petersburg, Russia.

Elevated expression of molecular chaperones in tumor cells is known to correlate with poor prognosis in most of cancers. Heat shock protein 70, Hsp70, provides the correct folding of newly synthesized or damaged polypeptides while Hdj1, Hdj2 and relative proteins assist to major chaperone in ATP-dependent manner. We studied how the knock-down of the abundant Hdj2 co-chaperone affect physiology of highly malignant C6 rat glioblastoma cells. To shift the level of the protein down we employed shRNA technology coupled with lentivirus infection. Knock-down of Hdj2 caused considerable changes in C6 cell physiology. C6shHdj2 cells demonstrated high migration activity and the ability to detach from substrate and form new colonies. These cells lose intercellular contacts mediated by N-cadherin as well as increased level of matrix metalloproteases in the cell medium in comparison with the parent C6 cells. Being injected into rat brain C6shHdj2 cells demonstrated elevated invasiveness and metastatic activity as well as dramatically reduced survival of tumor-bearing animals. Taking together our data prove the significant role of Hdj2 in tumorigenecity and metastasis.

ROLE OF BREACH OF REGENERATION IN STIMULATION MITOGENIC ACTIVITY AND INCREASE EXPRESSION p53. © *A. V. Pechersky*,[1] *V. I. Pechersky*,[1] *A. B. Smolyaninov*,[2] *V. N. Vilyaninov*,[3] *S. F. Adylov*,[2] *A. Yu. Shmelev*,[1] *O. V. Pecherskaya*,[1] *V. F. Semiglazov*.[4] [1]North-West State Medical University named after I. I. Mechnicov, St. Petersburg, Russia, [2]Pokrovsk bank of stem cells, St. Petersburg, Russia, [3]Army Medical Academy of S. M. Kirov, St. Petersburg, Russia, [4]N. N. Petrov Scientific-Research Institute of Oncology, St. Petersburg, Russia.

B a c k g r o u n d. After 35—40 years at people the decrease in a pool of pluripotent stem cells resulting in insufficiency of replenishment of cellular structure of cambial zones and, as a result, to incomplete replacement of the perishing old cells is observed. In reply surrounding epithelial and endothelial

cells, and also the macrophages, attracted with death of old cells, stimulate cells division of growth zones by the cellular growth factors (Pechersky A. V. et al., 2008).

Materials and methods. To 11 patients aged from 52 till 76 years with a cancer of a kidney, bladder, prostate gland of the III—IV stage of disease the chemotherapy or target therapy was carried out. To 4 patients of 60—82 years for restoration of regeneration it was carried out from 4 to 7 transfusions of mononuclear fraction of peripheral blood, same-gender and blood types with recipients.

Results. In 1 month after carrying out chemotherapy or target therapy after development of a leukopenia in 11 patients the level of the basic fibroblast growth factor (bFGF) increased on average by 1.74 times, at 4 patients from them the increase in level of the human vascular-endothelial growth factor (human VEGF-A) on average by 1.25 times was observed, the 3rd of them had an increase in the human epidermal growth factor (human EGF) on average by 1.13 times.

In 3—6 months after the last transfusion of mononuclear fraction of peripheral blood the maintenance of hemopoietic cells predecessors of $CD34^+$ in peripheral blood increased on average by 3.25 times (at 4 patients with 1 to 2—5 cells in 1 mcl). At 4 patients the level of the basic fibroblast growth factor (bFGF) decreased on average by 1.78 times, at 2 patients from them reduction of level of the human vascular-endothelial growth factor (human VEGF-A) on average by 1.48 times was observed, the 3rd of them had a reduction an human epidermal growth factor (human EGF) on average by 4.12 times. Decrease in levels of cellular growth factors naturally brought at all 4 patients in a buccal epithelium to decrease in an expression of p53 on average by 6.02 times, at the 3rd of them to decrease in an expression of bcl-2 on average by 60.0 times.

Conclusion. Excess stimulation of mitotic activity at people 40 years are more senior it is possible to lower to normal level by means of restoration of number of a pool of pluripotent stem cells by transfusion of mononuclear fraction of the peripheral blood from young donors of 18—23 years of one with the recipient blood types and a sex.

RECOGNITION OF RAT GLIOBLASTOMA CELLS IN VITRO AND *IN VIVO* BY HSP70 CHAPERONE-ASSOCIATED NANOPARTICLES.

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Hsp70 was earlier found to bind selected receptor structures including CD40 on cancer cells and we addressed the question whether the chaperone can be used for MR contrast enhancement of C6 rat glioblastoma cells. We constructed superparamagnetic iron oxide nanoparticles (SPIONs) that due to their unique magnetic properties may function both as magnetic resonance (MR) contrast agents, and can be used for thermotherapy. SPIONs were conjugated to the pure recombinant human Hsp70. A significant accumulation of the Hsp70-SPIONs but not the non-conjugated nanoparticles was observed by confocal microscopy within C6 cells. In intracranial model of glioblastoma Hsp70-SPIONs demonstrated higher negative contrast tumor enhancement in the T2-weighted MR images than non-modified SPIONs. Histochemical analysis of the brain sections confirmed that Hsp70-SPIONs localized in the tumor site and not in the adjacent normal brain tissues suggesting that the particles can be successfully employed as contrasting agents in MRI.

DIFFERENT ONCOGENIC PROPERTIES OF MDM2 ISOFORMES IN CANCER CELLS.

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Proto-onogene MDM2 is a well known principal negative regulator of tumor supressor p53. Besides targeting p53 for degradation via ubiquitinylation, MDM2 possesses a number of p53-independent oncogenic properties. The latter are being actively studied from both scientific and applied point of view. The average percent of wild type MDM2 overexpression in different tumors totals only about 7 %, whereas 30—90 % of clinical samples are characterized by the presence of various splice isoforms of MDM2. The most common are MDM2-A, -B, -C and -D splice isoformes. They occur predominantly in the late (III and IV) stages of tumor development and are usually associated with poor prognosis. So, we have focused our study on potential molecular mechanisms by which MDM2 splice isoformes contribute to oncogenesis and tumor progression in comparison with the full-length MDM2 protein. To do this, we constructed lentiviral vectors harboring MDM2-FL, -A, -B, -C and -D isoformes and subsequently infected the set of breast and lung cancer cell lines which differ by molecular taxonomy. We have assessed the influence of particular MDM2 isoforms on cell proliferation, motility and sensitivity to the Type I interferon treatment by various techniques, including colony formation assay, wound healing, apoptosis and Q RT-PCR of specific biomarkers. We established significant differences between specific isoforms of MDM2 in respect to their effect on proliferation and interferon sensitivity. To gain insights into the molecular mechanisms of these phenotypes, we performed a proteomic analysis of MDM2-interacting proteins coupled with mass-spectrometry. Collectively, our results propose an existence of isoform-specific interacting partners that mediate oncogenic effects of MDM2.

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SIGNALING PATHWAYS INVOLVED IN MAINTENANCE OF SELF-RENEWAL AND PLURIPOTENCY OF MOUSE EMBRYONAL STEM CELLS.

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Study on the molecular mechanisms underlying self-renewal, pluripotency and differentiation of mouse embryonal stem cells (mESCs) identified several key signaling pathways: LIF/STAT3, FGF/Ras/MAPK, Wnt/β-cathenin etc. Unlimited self-renewal of mESCs is provided by a partial dysfunction of p53/p21Waf1 pathway that allow them do not stop in G1 and thereby to avoid an impetus to differentiation. In spite of the lack of G1-checkpoint control, the program for recognizing and repairing DNA defects (DDR signaling) is functional in mESCs. We used several agents with different mechanisms of p53 and/or p21Waf1 activation to study a transition

from self-renewal to early differentiation: gamma-irradiation, nutlin, histone deacetylasel inhibitors, AICA-riboside. The data obtained show that only those agents, which reactivate p53 and cause accumulation of its target p21Waf1protein, are capable of restoration of G1/S checkpoint and induction of differentiation that in turn is accompanied by down-regulation of pluripotency genes *oct-4* and *nanog* and up-regulation of endoderm-specific genes.

We found that the LIF/STAT3 signaling pathway can contribute to the maintenance of self-renewal and pluripotency of mouse ESCs by suppressing mTOR pathway. The mTOR signaling is known to be involved in proliferation, cell growth, translation regulation and cell metabolism. When LIF ligand is withdrawn from culture medium, the mTOR activity rapidly increases as detected by phosphorylation of its targets — ribosomal protein S6 and translation factor 4EBP1. In turn, suppression of STAT3 phosphorylation on Tyr-705 by a specific small molecule WP1066 also activates phosphorylation of the mTOR target S6 ribosomal protein. LIF removal strongly activates ERK activity indicating that ERK can be involved in either direct phosphorylation of mTOR or phosphorylation of an upstream negative regulator of mTOR — TSC2 protein. mTOR activation is accompanied by a decrease of pluripotent gene expression *Oct-4*, *Nanog*, *Sox2* and by an increase of *fgf5* gene expression — an early marker of neuronal commitme. Together, these data indicate that upon LIF withdrawal mouse ESCs undergo a transition from LIF/STAT3-supported pluripotency to FGF/Erk-committed differentiation mediated through activation of mTOR signaling.

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HUMAN LYMPHOCYTES INDUCE APOPTOSIS AND NECROPTOSIS IN DIFFERENT CANCER CELL LINES VIA CONTACT AND SECRETORY MECHANISM.

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In this work we study mechanisms of cytotoxic activity of human lymphocytes, activated via incubation with IL-2 cytokine for 6 day. Activated lymphocytes kill several cancer cell lines using different mechanisms. Joint incubation with K-562 cancer cells leads to lysis of cancer cells via FasL-Fas contact mechanism. During this coincubation lymphocytes secrete in the conditioned medium complex of two proteins Tag7—Hsp70, that possess cytotoxic activity to several other cancer cell lines, including L-929 cells. In this case Tag7—Hsp70 complex induces cancer cell death via interaction with TNFR1 receptor. In both cases cancer cells are dying via programmed cell death with activation of several mechanisms — caspase-dependent apoptosis after 3 h of incubation and RIP1-dependent necroptosis after 20 h of incubation.

NOVEL SMALL-MOLECULE INHIBITORS OF MDM2-P53 PROTEIN-PROTEIN INTERACTION.

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The major tumor suppressor protein p53 is a transcriptional master regulator that controls cell cycle arrest, senescence, apoptosis, autophagy, DNA repair and metabolism (Vousden, Prives, 2009). In the absence of stress signals, p53 is inactivated by E3 ubiquitin ligase MDM2 that targets p53 for degradation (Momand et al., 1992; Oliner et al., 1993; Kubbutat et al., 1997). Thus, p53 stabilization and activation in malignant cells is a very promising target for anticancer therapy. Small molecule candidates selected from *in silico* analyses were screened for the ability to stabilize p53 in cancer cells using a GFP-based p53-responsive reporter plasmid. Two isogenic human osteosarcoma cell lines U2OS^{p53+} and U2OS^{p53-} (stably expressing shRNA knockdown plasmid of p53) were used for evaluation of specificity of small molecules towards p53. To assess the effect of compounds on p53 stabilization the protein levels of p53 in cell lines after treatment with chemical compounds were analyzed by western blot. Experiments have shown that least three selected compounds stabilized p53 comparable to Nutlin-3, a well known p53 activator. We simultaneously examined the p53 mRNA level after treatment with small molecule activators of p53 and Nutlin-3, which was used as control. Real-time PCR assay showed equal levels of p53 mRNA expression after treatments, indicating that the compounds affect p53 on the protein level likely by modulating its protein stability. The tumor suppressor p53 functions as a transcription factor by modulating the expression of several target genes (p21, PUMA, Bax etc.), whose products, in turn, regulate cell cycle, apoptosis, etc. Real-time PCR data have shown that two compounds induced the expression of p53 target genes (p21, PUMA and BAX). A cytotoxic effect of these compounds was evaluated based on the colony formation assay using U2OS^{p53+} and U2OS^{p53-} cell lines. Collectively, our results demonstrated that two compounds specifically activate p53 on the protein level and have no cytotoxic effect on U2OS^{p53-} cell line. In sum, these two compounds are chosen for in vivo preclinical studies.

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THE ROLE OF METHYLTRANSFERASE SET7/9 IN PARASPECKLE AND SAM68 NUCLEAR BODIES FORMATION.

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Paraspeckles are a relatively new class of subnuclear ribonucleoprotein bodies found in the interchromatin space that form around the long non-coding RNA (lncRNA) nuclear paraspeckle assembly transcript 1 (Neat1) together with RNA-binding proteins [1]. Sam68 (Src-associated substrate during mitosis of 68 kDa) nuclear body is another type of nuclear body located adjacent Cajal bodies.

Using MALDI-TOF mass spectrometry we identified a number of proteins which interact with methyltransferase Set7/9. Set-domain containing lysine methyltransferase (KMTs) catalyzes site-specific methylation of lysine residues in histone and non-histone substrates. This modifier plays a crucial role in regulation of transcription, heterochromatin formation, inactivation of X-chromosome and response to DNA damage.

Among indentified proteins we discovered a number proteins essential for paraspeckles formation. In other hand we found that Sam68 RNA-binding protein also interacts with Set7/9. Sam68 is one of the STAR RNA-binding proteins in-

volved in the regulation of the cell cycle, cell signaling, RNA biogenesis, and alternative splicing. It also mediates export of un-spliced viral RNA as well as plays role in oncogenesis [2].

The domain structure of Sam68 includes several regions with known function(s): the KH domain is required for binding RNA, proline- (N-terminus) and tyrosine-rich (C-terminus) regions are required for protein-protein interactions. The function of another Sam68 domain rich in Arg and Gly (RG domain) has not been fully understood yet. It has been shown that arginines the RG domain are methylated by arginine-specific methyltransferase PRMT1 [3, 4]. Hypomethylated Sam68 is partially re-localized from the nucleus to cytoplasm [4]. This may indicate the regulatory role of methylation in life cycle of this multifunctional protein. Furthermore, expression and localization of Sam68 correlates with the occurrence and degree of tumorigenicity. Overexpression of Sam68 is associated with poor prognosis of patients with prostate, breast, kidney and neuroblastoma cancers [5—7].

By using a proteomic approach, we demonstrated that the RG-rich domain of Sam68 interacts with Set7/9 both *in vitro* and *in vivo*. Furthermore, we have identified a specific domain of Set7/9 responsible for this binding.

These results warrant further investigation of the biological role of the interaction between KMT Set7/9 and the RNA-binding protein Sam68 as well as the role of Set7/9 in regulation of paraspeckle function and formation in human cancer cells.

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NOVEL SUBSTRATES OF HIGHLY CONSERVED PROTEIN COP1.

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In 2004 COP1 was characterized as p53 E3-specific human ubiquitin ligase according to *in vitro* studies. Later oncogenic activity of COP1 and correlation of its overexpression with level of p53 protein in clinical tumor samples and cell lines were shown. High expression level of COP1 has been described in 25 of 32 cases of breast carcinomas and in 76 out of 171 cases of ovarian carcinomas. High expression level of COP1 was described in hepatocellular carcinoma cell lines (PLC, Hep3B and HepG2, Huh7). In 40 of 55 cases of pancreatic cancer overexpression of COP1 was also noted. Some types of leukemia, melanoma, breast, lung and prostate cancer contain focal deletions of COP1. COP1 overexpression was also found in non oncological diseases like Duchenne muscular dystrophy, ischemic cardiomyopathy and juvenile dermatomyositis. Similar to other p53-related E3-ubiquitin ligases COP1 has different substrates. The selection of target for ubiquitination depends on cell type or differentiation stage. Among the substrates of COP1, besides p53 are: c-Jun, ETV1, ACC, TORC2, FOXO1 and C/EBP?, FIP200. To identify novel proteins that interact with COP1 we applied proteomics. Proteins co-immunoprecipitated with COP1 were analysed by MALDI-TOF Mass spectrometry. To achieve this, COP1-3xFlag expression vector was generated and transfected into HEK 293T cells by calcium phosphate transfection method. Protein complexes were immunoprecipitated with anti-FLAG beads. Bound proteins were then separated by 1D SDS-PAGE gel electrophoresis and analyzed by means of ESI-LC/MS/MS mass spectrometry. Subsequent bioinformatics analysis of the interacting proteins was employed. About 25 % of identified proteins were attributed to the cytoskeleton proteins, almost 20 % of proteins had known role in metabolism. According to the literature data, it was not surprising to find several proteins involved in the lipid metabolism. Finally, a significant groups of interactants play role in transcription, cell adhesion, apoptosis, protein modification and transport. It is important to note that several COP1-bound proteins can be strong regulators of cell cycle in G2/M transition. Our data can be used for the subsequent studies of previously unknown roles of COP1 in different diseases. Acknowledgements: This work was funded by grants from RSCF (№14-15-00816) and Program in MCB at RAS.

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INVESTIGATION OF THE BEHAVIOR OF NEW SILICA-BASED NANOPARTICLES IN CULTURES OF C6 RAT GLIOBLASTOMA CELLS.

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Nanoparticles become an emerging tool for drug delivery in oncology. Newly synthesized monodisperse spherical mesoporous silica particles (MSMSP) are suggested to possess several advantages as drug carriers and as instruments for theranostics because they can be easily functionalized and may be observed in cells and tissues as bright sport. The aim of this work was to study the effect of these particles with different structure and size on cancer cells. In our experiments we used two types of MSMSP (S1 — 150 nm, S2 — 450 nm diameter) containing 3.1 nm diameter cylindrical mesopores almost completely filled with $Gd^2O^3 : Eu^{3+}$ (5 mole % Eu^{3+}). The core-shell S4 and S5 particles were the particles S1 and S2, respectively, additionally covered with the 50 nm mesoporous SiO_2 shell. C6 rat glioblastoma were incubated with 100 or 500 MSMSP per cell for 6, 24 and 48 hrs. Following washing the cell viability was measured using method of Mossman with MTT staining. The results show that the cell viability and growth rate were not affected by particles of both sizes and irrespective of whether they were covered with silica or not and taken in two different concentrations suggesting that their application in organism is safe. When incubated with C6 cells for 6 hrs particles of both sizes according to confocal microscopy in reflected light (488 nm) gave green speckles inside the cells; the amount of cells targeted by MSMSP of S2 type was maximal as compared with the other probes. Interestingly, the cell-penetrating ability inside immortalized mouse 3T3 fibroblast was substantially lower that shows probable targeting directory of the MSMSP. The work on functionalized particles is now in progress.