SESSION 10
LATE BREAKING NEWS

THE ANTIBIOTICS, DOXYCYCLINE (Dox), CHLORAMPHENICOL (Cp) AND GENETICIN (G418), CAUSE HIGH ERROR RATES IN SELENOCYSTEINE (Sec) INSERTION IN MAMMALIAN CELLS

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Antibiotics such as Dox, Cp and G418 are widely used in mammalian cell culture and Dox and Cp are used to treat infections in humans. We examined the effects of these antibiotics on Sec insertion into selenoproteins in murine EMT6 breast cancer cells and found that they interfered dramatically with selenoprotein synthesis. After treatment, the expression levels of thioredoxin reductase 1 (TR1), glutathione peroxidase 1 (GPx1) and glutathione peroxidase 4 (GPx4) were analyzed by ^{75}Se and western blot. All three antibiotics induced the expression of TR1 and GPx4 by western blot, whereas ^{75}Se showed a decrease in TR1 and GPx4 expression in treated cells. The expression of GPx1 in treated cells decreased in both western blot and ^{75}Se. Recombinant TR1, GPx1 and GPx4 were purified from treated cells, specific activity measured, and MS/MS analysis determined the amino acids that were inserted in place of Sec and, in the case of TR1, whether the protein was also truncated at the penultimate (UGA) codon. TR1 activity was significantly reduced in the presence of antibiotics, while GPx1 activity was only slightly reduced. The results of MS/MS analysis revealed that the Sec-containing form of TR1 decreased, whereas the Arg-containing and truncated forms increased. Antibiotic-specific misinsertion of Cys and Trp were also detected. Misinsertion of Arg in place of Sec was also observed in GPx1 and GPx4. TR1 was the most affected and GPx1 was the least affected by the translation errors. These observations were consistent with the differential use of two Sec tRNA isoforms and their distinct roles in supporting accuracy of Sec insertion into selenoproteins. Furthermore, our study raises the possibilities that prolonged usage of these antibiotics in treating human infections and in inducing transgenic mouse models may cause selenoprotein deficiencies.

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THIOREDOXIN REDUCTASE 1 PROTECTS AGAINST HEPATOCARCINOGENESIS VIA CONTROL OF CELLULAR REDOX HOMEOSTASIS

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Thioredoxin reductase 1 (TR1) is one of the major redox regulators in mammalian cells. Since cancer cells suffer from oxidative stress and TR1 acts in protecting normal cells from oxidative stress, this enzyme has been proposed to have a role in cancer prevention. However, other studies have suggested that TR1 promotes cancer development. It is overexpressed in many cancers and cancer cell lines and TR1 has been targeted by a number of potent inhibitors and anticancer drugs that reduce its activity, which in turn can reverse cell morphology and other cancer-like properties. These observations have led to the proposal that TR1 is a prime target for cancer therapy. To determine the role of this selenoprotein in hepatocellular carcinoma development, we examined tumor incidence in livers of mice with hepatocyte-specific knockout of TR1 treated with the carcinogen, diethylnitrosamine (DEN). TR1-deficient livers manifested ~90% tumor incidence compared with ~16% in control mouse livers. We also observed upregulation of another selenoenzyme, glutathione peroxidase 2, and components of the glutathione system, as well as other NF-E2-related factor 2 (Nrf2)-regulated antioxidant genes. Overall, the study shows that TR1 protects against chemically induced hepatocarcinogenesis via the control of the cellular redox state, whereas its role in promoting this type of cancer may be minimal. Further, we have examined the expression of selenoproteins (e.g., TR1, glutathione peroxidase 4, glutathione peroxidase 1, and other antioxidant proteins (e.g., glutaredoxin, peroxiredoxin, su-

peroxide dismutase and catalase) in human liver and lung cancer specimens, revealing the possible interplay between antioxidant selenoproteins and other antioxidant proteins in two distinct organs and cancers. These results may provide relevant information for assessing human clinical trials. This research was supported by the Intramural Research Program of the NIH, NCI, CCR.

INCREASING DIETARY SELENIUM ACCELERATES PROGRESSION OF SELECT MESOTHELIOMA TUMORS BY RAISING CELLULAR REDUCING CAPACITY THAT PROMOTES ERK ACTIVATION

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The effects of selenium supplementation on tumor progression may vary with different types of cancers. To determine the in vitro outcome of increasing dietary selenium on the progression of malignant mesothelioma (MM), four different MM cell lines arising from asbestos or erionite instillation in mice were cultured in media containing increasing amounts of sodium selenite (30, 50, and 80 nM final concentration). Surprisingly, increasing selenium did not exert anticancer effects and actually increased density dependent proliferation and mobility for certain MM cell lines (CRH5 and EKKH5) but not others (AB12 and AK7). Evaluation of pro-growth signaling molecules revealed that ERK phosphorylation was sensitive to increases in selenium in CRH5 and EKKH5 but not AB12 and AK7 cells. Stable expression of a dominant negative mutant ERK eliminated the effects of increasing selenium. Because ERK is redox sensitive, we compared the redox status of the selenium sensitive and insensitive MM cells in terms of glutathione levels and reduction of exogenous hydrogen peroxide. Increasing selenium led to higher reducing capacity in CRH5 but not AB12. Addition of the reducing agent N-acetyl-cysteine eliminated the effects of selenium on ERK activation, proliferation, and mobility in MM cell lines. Finally, in vivo studies were conducted in which mice were fed diets containing increasing levels of selenium (0.08, 0.25, 1.0 ppm) and then injected with CRH5 and AB12 MM cells. Higher selenium intake led to increased tumor progression for CRH5 but not AB12 MM cells, and N-acetylcysteine treatment eliminated the effects of increasing selenium in CRH5 tumor progression. Overall, these findings suggest that the effects of dietary selenium on MM tumor progression depend on the extent to which the cancer cells convert increased selenium into a stronger reducing capacity, and some MM tumors actually benefit from increased selenium intake.

SIMULTANEOUS DETERMINATION OF SELENIUM AND REDOX PERTURBATION DEPENDENCES OF NFκB, Nrf2 AND HIF ACTIVATION PATTERNS AT SINGLE-CELL RESOLUTION

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Redox regulation, i.e. regulation of cellular signaling pathways via reversible reduction/oxidation of key cellular factors, is an important and rapidly emerging concept. Because of fast, transient and complex events, this is also a technically difficult area to study. Key components in redox regulation are the thioredoxin- (Trx) and glutathione- (GSH) dependent systems. These pathways may in turn modulate the activities of redox regulated transcription factors (e.g NFκB, HIF, Nrf2, p53, AP1, Oct4). We here describe a newly developed methodology for the determination of three major transcription factor activities, known to be modulated by redox pathways and selenium status. Our major tool is a single reporter vector that expresses three separate fluorescent proteins, each coupled to unique response elements for NFκB, Nrf2 or HIF, respectively. With this new methodology it becomes possible to study the regulation of these transcription factors by the Trx- as well as the GSH-systems, or in cells grown with different selenium status. Using several growth and treatment conditions, we report time-, concentration- and cell-specific activation patterns for all these three transcription factors. Importantly, single cell analyses also reveal stochastic activation patterns of NFκB, Nrf2 and HIF within very same cell populations; such heterogenic responses are not revealed using conventional analyses based on whole cell populations. To conclude, the new methodology reported here enables a characterization of key redox regulated transcription factors at a hitherto unsurpassed level of detail. In combination with relevant model systems, the methods developed here may thus help to further our understanding of key events in redox control for cellular function in health and disease, including the impact of cellular selenium status or individual selenoproteins. We also suggest that the tool developed here could be further tailored for detection of additional response elements.

HYPOXIA AFFECTS SELENOPROTEIN EXPRESSION

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Selenoprotein expression is tightly regulated by selenium (Se) availability, transcription of selenoprotein genes and post-transcriptional mechanisms controlling selenocysteine insertion. A number of human diseases are characterized by low Se status, e.g., sepsis, trauma, cancer, autoimmune and infectious diseases. The aforementioned diseases are uniformly characterized by reduced perfusion, i.e., a limited oxygen supply. This common motif may negatively affect selenoprotein biosynthesis and cause a systemic Se status decline. We thus hypothesized that low oxygen supply impairs Se metabolism and selenoprotein expression at the transcriptional level or during translation.To this end, human hepatocarcinoma HepG2 cells were exposed to regular or low oxygen conditions (i.e. 20% and 1%). Cells were harvested and selenoprotein expression levels were determined by qRT-PCR, Western blot analyses and suitable enzyme assays, respectively. These analyses were expanded to central factors involved in Se metabolism and selenoprotein biosynthesis. Selenoprotein P in medium declined by approx. 70% as well as activities of DIO1, GPX or expression of SELS under hypoxia. Notably, effects were specific and not observed for all selenoproteins alike, e.g., GPX4 even increased under hypoxic conditions. When testing central factors involved in selenoprotein biosynthesis, mRNA expression levels of PSTK, SEPSECS, EFsec and SBP2 decreased under hypoxia. Respective reporter gene analyses supported these findings and showed down-regulation of promoter activities of a set of selenoprotein and Se metabolism-related genes confirming that hypoxia elicits a strong negative effect on the selenoprotein biosynthesis machinery and transcription of certain selenoproteins. Collectively, our finding may help to explain the disease-associated decline in Se status and selenoprotein expression as observed in severe diseases and highlight oxygen supply as a common and strong selenoprotein expression modifier.

ANTIOXIDANT AND HEPATOPROTECTIVE EFFECT OF NEWLY SYNTHESIZED OF SELENOORGANIC COMPOUNDS

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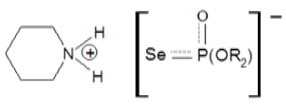
The aim of our investigation was synthesis and develops of new selenoorganic compounds which will used in medicine, particular for correction of oxidative and toxic demerged of hepatitis of difference genesis. We were synthesis and proved structure of more 4 -th dozen new selenoorganic compound. After that the syntheses compounds had been tested on antioxidative and hepatoprotective activity. But the way during the experiment screening some new synthesis compounds were utilized for high toxicity it has low activity. The next step we have established acute toxicity (LD_{50}) of new selenoorganic compounds and its related there structure and with toxicity a biological activity. In this work we present the organoselenium compound with a quaternary nitrogen atom (PSP piperidine (dialkilselenophosphat), who showed the most expressed antioxidant and hepatoprotective effect.



Piperidine (dialkilselenophosphat)

Experiments were carried out on models of acute toxic hepatitis phosphorus genesis. As we know,acute phosphoric intoxication (API) increases the processes of free-radical oxidation, and activation of parameters of chemiluminescence and depression of antioxidant system in rat liver. The injection of selenoorganic compounds on API increases the survival rate reduction chemiluminescent indicators, normalization of lipid peroxidation and the induction of antioxidant enzymes: SOD, GP, GR and CAT. To assess the functional liver condition, we determined the enzyme activity of hepatocytes cytolysis ALT, AST and LAP. Injection to animals with acute phosphorus intoxication selenium compounds of PSP has restored activity listed above enzymes of liver.

Conclusions: Organic selenium PSP, which containing in the structure of cationic atom of nitrogen has more antioxidant and hepatoprotective effects. In the present time organic selenium PSP will be clinical trials.