SESSION 8
SELENIUM AND HUMAN HEALTH II

SELENOPROTEINS IN THE BRAIN

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Selenium effects in brain remained ill-defined and disputed until gene targeting experiments investigating the roles of glutathione peroxidase 1 (GPx1) and selenoprotein P (SePP) revealed that the lack of selenoproteins in mouse brain leads to neurological phenotypes. Almost all selenoproteins and several selenoprotein biosynthetic factors, as well as SePP-receptors, have been genetically inactivated painting a picture of specific roles of selenoproteins in the brain. Most work has focused on neuronal selenoproteins. In fact, neurons require selenoproteins for normal development and function, but differences exist among neuronal cell types. For example, parvalbumin-positive GABAergic interneurons in cerebral cortex and hippocampus are particularly sensitive to disturbed selenoprotein expression. Less is known about the roles of selenoproteins in glial cell types. While epileptic seizures are a plausible result of GABAergic interneuron dysfunction, the movement phenotypes of selenoprotein-deficient mice remains less well explained. More recently, two syndromes of selenoprotein biosynthetic defects (mutations in SECISBP2 and in SEPSECS) have underlined the essentiality of selenoprotein expression in the human brain. It has been proposed that impaired selenoprotein expression may exacerbate human neurodegenerative disease. Whether this mechanism plays a role in Alzheimer's or Parkinson's disease remains to be shown - alternatively, impaired selenoprotein expression may present with similarities to both diseases, but may represent a distinct pathological entity.

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STRATEGIES FOR SELENIUM TRANSPORT AND REGULATION

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Selenium is an essential nutrient but it becomes a toxin when present in excess. The mechanism for maintaining whole-body selenium within its physiological range resides in the liver. Competition for selenide between synthesis of selenocysteine, leading to retention of selenium in the body, and methylation, leading to selenium excretion, determines its fate. Once it is in the form of selenocysteine attached to tRNA, liver selenium is incorporated into intracellular selenoproteins or into selenoprotein P (Sepp1) for export to other tissues. Extra-hepatic tissues vary in their selenium needs, necessitating a delivery mechanism that can apportion the element accordingly. That mechanism is endocytosis of Sepp1 mediated by apolipoprotein E receptor-2 (apoER2). Tissues that export selenoproteins, such as testis and bone marrow, express greater amounts of apoER2 than other tissues. The brain, which requires selenium for its viability, takes up Sepp1 at the blood-brain barrier (BBB) via apoER2-mediated endocytosis. The brain then retains its selenium by incorporating it into Sepp1 in glial cells and delivering it to neurons via the Sepp1-apoER2 pathway-all inside the BBB. Kidney proximal convoluted tubule (PCT) cells take up a filtered form of Sepp1 via megalin-mediated endocytosis and use its selenium to synthesize glutathione peroxidase-3 for export to basement membranes in many tissues. In addition to the receptor-mediated mechanisms, selenium can also be transported in small-molecule form. However, the selenium intake of the animal must be very high for this mechanism to be effective. Thus, recent investigations have uncovered strategies used to regulate the selenium content of the body and to ensure its appropriate delivery to tissues.

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MECHANISMS BY WHICH GPx-1 CAN AFFECT CANCER RISK AND OUTCOME

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GPx-1 is the first and most extensively characterized selenoprotein, being implicated in cancer etiology by several different lines of evidence. As an antioxidant capable of reducing reactive oxygen species, GPx-1 may reduce reactive oxygen species (ROS) that either can result in DNA damage or alter signaling pathways that are responsive to ROS, such as those that mediate the repair of DNA damage. This concept is supported by data indicating that elevated GPx-1 expression can not only reduce the levels of DNA damage but stimulate the expression of proteins that function in DNA repair, such as H2AX and Chk2. These same functions of GPx-1 that may reduce cancer risk may be detrimental if operative in

existing tumors, and this is supported by data indicating that higher levels of GPx-1 are associated with a higher Gleason Score, a histopathological indicator of poor clinical outcome for prostate cancer.

Among the strongest evidence for a role for GPx-1 in cancer is data indicating that different GPx-1 alleles are associated with increased risk of several cancer types. Most often, DNA from individuals that encodes a GPx-1 with a leucine located at position 198 are at elevated risk of disease. Using human MCF-7 cells that express distinct GPx-1 alleles, we show that there is an interaction between the codon 198 polymorphism and another variation resulting in a variable number of alanines in the amino terminus of the protein. These variations can impact the distribution between GPx-1 between the cytoplasm and the mitochondria, and likely result in significant biological consequences. Using cells expressing GPx-1 exclusively located in the mitochondria due to the addition of a mitochondrial localization sequence, it is shown that primary sequence and cellular location can affect GPx-1 function and energy metabolism. Collectively, these data support the continued investigation of the role the GPx-1 selenoprotein plays in cancer risk, prognosis and cellular homeostasis.

SELENIUM-DEPENDENT PATHWAYS OF RESOLUTION OF INFLAMMATION

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Selenium (Se) and selenoproteins impart anti-inflammatory functions. Our studies have demonstrated the ability of Se to suppress the activation of a key redox-sensitive transcription factor, NF-kB, and its downstream target genes, including proinflammatory cytokines, chemokines, and enzymes in immune cells, particularly macrophages. In addition, we found that changes in the cellular redox status via the expression of selenoproteins activated metabolic pathways to enhance the production of cyclopentenone prostaglandins (CyPGs) that are endogenous metabolites derived from arachidonic acid, an abundant cellular polyunsaturated fatty acid. Such selenoprotein-dependent metabolomic changes resulted in polarizing pro-inflammatory (M1) macrophages more towards pro-resolving or alternatively activated (M2) macrophages. The effect of macrophage polarization towards "wound healing" or pro-resolving phenotypes by Se was seen in diverse models of inflammation, such as DSS-induced colitis, infection of helminth parasites, and transplantation of leukemic stem cells (LSC). In these models, selenoprotein expression was essential for timely resolution. While macrophage expression of selenoproteins clearly played a role in resolving DSS-induced inflammation in the colon and enhanced apoptosis of LSCs, expression of macrophage selenoproteins greatly increased helminth clearance suggesting wound healing. Se treatment of LSCs, but not normal hematopoietic stem cells, increased cellular oxidative stress leading to selective apoptosis of the former cell type. Cellular mechanisms of wound healing and apoptosis will be discussed. The implications of these studies on future clinical trials with selenium will be also discussed.

IDENTIFICATION OF SELENOPROTEIN P (Sepp1) AND APOLIPOPROTEIN E RECEPTOR 2 (apoER2) BINDING INTERACTION SITES

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Sepp1 transports selenium from the liver to other tissues. ApoER2, a member of the low-density lipoprotein receptor family, has been demonstrated to facilitate cellular uptake of Sepp1. Sepp1 binding to the extracellular region of apoER2 is believed to initiate endocytosis and supply selenium to cells. The extracellular region of mouse apoER2 consists of four types of protein modules. These include the ligand binding repeats (LBR), the epidermal growth factor repeat, the YWTD b-propeller module and the O-linked sugar module. The LBR has three disulfide bonds and a calcium-binding site formed by highly conserved acidic amino acid residues. Sepp1 binding was assessed in HEK293T cells overexpressing mouse apoER2-GFP fusion proteins. Sepp1 was introduced by incubating cells in culture media containing mouse serum. Following washes in PBS, cells were harvested for protein and Sepp1 binding was analyzed by SDS/PAGE and western blot analysis. Sepp1 binding was detected in apoER2 overexpressing cells but not in the control cells without apoER2 expression. The Sepp1-binding region within apoER2

was mapped to the five LBR modules. Using further apoER2 deletion analysis, we examined potential Sepp1-binding sites. In addition to full-length Sepp1, isoforms of varying length have been identified in rat plasma. These isoforms contain an identical N-terminus but terminate at the positions corresponding to termination before the 2nd, 3rd and 7th selenocysteine codons. We determined that the $Sepp1^{\Delta 240\text{-}361}$ isoform, which contains a single selenocysteine, did not bind to apoER2-GFP. HEK293T cells overexpressing V5-tagged Sepp1 constructs secreted the various mutant isoforms into the culture medium. We are currently investigating the residues involved in Sepp1-apoER2 binding and interactions with other apoER2 binding proteins and their uptake mechanisms.

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SELENOPROTEIN P MEDIATED MODIFICATION OF SYNAPTIC PHYSIOLOGY AND PLASTICITY

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Selenoprotein P (Sepp1) is an extracellular protein with multiple selenocysteine residues involved in interorganal transport of selenium. Association of Sepp1 with pathology in neurodegenerative disorders as well as neurological impairments in Sepp1 knockout (Sepp1KO) animals demonstrate importance of Sepp1 for brain function. However, the direct actions of Sepp1 in brain and mechanisms of selenium delivery are poorly understood. We have been investigating the expression of Sepp1 in human postmortem brain, including Alzheimer´s and Parkinson´s brain. Previously we described expression of Sepp1 in neurons of the cortex, substantia nigra and putamen, as well as cuboidal cells of the choroid plexus. We found Sepp1 to be associated with both Alzheimer´s and Parkinson´s brain pathology. We are also investigating the role of Sepp1 in synaptic physiology using Sepp1KO mice. Deletion of Sepp1 results in bidirectional changes in synaptic plasticity. Deficits observed in Sepp1KOs in long-term potentiation (LTP), a cellular model for learning and memory, can be reversed by introducing Sepp1 directly in the hippocampus, indicating a direct action of Sepp1 on neuron physiology. Altogether these studies demonstrate the importance of Sepp1 in maintaining synaptic integrity and plasticity.

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