## *SESSION 13*
*BIOLOGY AND FUNCTION OF SELENOPROTEINS I*

# GLUTATHIONE PEROXIDASE 2 IN INTESTINAL NEOPLASIA

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The gastrointestinal glutathione peroxidase (GPx2) appears to have a dual role during colorectal carcinogenesis. While it promoted growth of xenografted tumor cells, it protected mice from colon cancer in a model of inflammation-triggered carcinogenesis. The observed anti-carcinogenic effect of GPx2 was mainly based on its anti-inflammatory role during dextran sodium sulphate-induced colitis. Therefore, we aimed to analyse the effect of GPx2 in a mouse model mimicking sporadic colorectal cancer. GPx2-knockout (KO) and wild-type (WT) mice were adjusted to an either marginally deficient (-Se), adequate (+Se), or supranutritional (++Se) selenium status and were treated six times with azoxymethane (AOM) to induce tumor development. In this model, the number of tumors and dysplastic crypts was significantly lower in GPx2-KO than in respective WT mice. This may be explained by a higher number of basal and AOM-induced apoptoses in GPx2-KO mice that eliminate damaged or pre-malignant epithelial cells. In WT dysplastic crypts GPx2 was up-regulated in comparison to normal crypts which might be an attempt to suppress apoptoses. In contrast to the lower tumor number in GPx2-KO mice, tumor size was larger in GPx2-KO mice of the +Se group. Furthermore, GPx2-KO mice had higher numbers of infiltrated inflammatory cells in their intestinal mucosa and more plasma TNF-α than WT mice. This indicates that GPx2-KO mice are characterised by a low-grade inflammation, which might provide a tumor-promoting environment and, thus, could explain larger tumors in GPx2-KO mice. In conclusion, the role of GPx2 and presumably also of selenium during colorectal carcinogenesis obviously depends on the involvement of inflammation.

# SYSTEMIC INACTIVATION OF THE ALox15 GENE DOES NOT RESCUE GPx4 DEFICIENT MICE FROM EMBRYONIC LETHALITY

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Glutathione peroxidases (GPx) are catalytically active selenoproteins that reduce organic and inorganic hydroperoxides to the corresponding alcohols at the expense of reduced glutathione. Among the GPx-isoforms phospholipid hydroperoxide glutathione peroxidase (GPx4) is unique because of its capability of reducing complex peroxylipids even if they are incorporated in biomembranes and lipoproteins. GPx4 is a multifunctional protein that has been implicated in anti-oxidative defense, gene expression regulation, programmed cell death, spermatogenesis and embryonic brain development. Beside its catalytic activity GPx4 also functions as structural protein in sperm development. Homozygous GPx4 knockout mice are not viable and die in utero by midgestation (E7.5). However, the exact molecular reasons for intrauterine lethality are not completely understood. To explore whether the lack of catalytic function or the deficiency of the structural protein is the dominant reason for embryonic lethality we created knock-in mice (mutGPx4), which express a catalytically inactive GPx4 mutant (Sec46Ala). As homozygous GPx4 knock-out mice the homozygous knock-in animals are not viable and undergo embryonic resorption at E7. Heterozygous knock-in mice are viable, fertile and do not show major phenotypic alterations. Systemic Alox15 deficiency did not rescue the GPx4 knock-in mice from embryonic lethality. In fact $Alox15^{-/-}$+mut$GPx4^{+/+}$ mice also undergo embryonic lethality. This data suggest that the lack of catalytic activity is the major reason for the embryonic lethality of GPx4 deficiency and that systemic inactivation of the Alox15 gene does not rescue the lethal GPx4 deficient phenotype.

# ROLE OF THE SELENOPROTEIN THIOREDOXIN REDUCTASE-1 IN COORDINATING DIVERSE METABOLIC ACTIVITIES

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The thioredoxin (Trx) and glutathione (GSH) pathways are the two major NADPH-dependent reductase systems in cells. These systems are highly conserved, such that most species contain at least one, and often both, systems. Nevertheless, drugs that systemically interfere with either the Trx- or the GSH-pathway are generally well tolerated in adult patients or animals, suggesting that disruption of either system alone is benign. Similarly, mice lacking glutathione reductase are healthy. Thus, it was perhaps surprising that constitutive genetic disruption of nearly any component of the Trx system in mice resulted in embryonic lethality. To investigate this, we developed a conditional-null allele of the thioredoxin reductase 1 gene (*Txnrd1*), which we can disrupt in specific cells of post-embryonic mice (1). Our investigations have revealed that reductase-based activities of the GSH- and Trx-systems are robustly redundant; however to replicate DNA, cells require that at least one of the two systems be at least partially functional (2,3). We also found that, outside of these reductase-based activities, disruption of *Txnrd1* results in a metabolic switch that broadly impacts redox-, energy-, and drug-metabolism pathways (4,5). Our studies are revealing unexpected integration of diverse metabolic activities in cells, and suggest a central role for thioredoxin reductase-1 in coordinating the gene regulation that underlies this process. This activity is not complemented by the GSH system, which lends insights into why the Trx-system is essential in embryogenesis.

**References:**

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# EFFECTS OF Sep15 AND/OR TR1 DEFICIENCY ON CANCER PHENOTYPE OF MOUSE COLON CARCINOMA CELLS

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The cancer preventive properties of the essential nutrient selenium are partly mediated through selenoproteins. This study investigated the effect of individual and combined down-regulation of the 15 kDa selenoprotein (Sep15) and thioredoxin reductase 1 (TR1) in mouse colon carcinoma CT26 cells. shRNA knockdown of either Sep15 or TR1 inhibited anchorage-dependent and -independent cell growth, and lung metastasis. Surprisingly, double knockdown of both Sep15 and TR1 reversed the effects seen in single knockdowns. FACS analysis revealed that the significant G2/M cell cycle arrest in shSep15 cells was no longer observed in cells lacking both Sep15 and TR1. To investigate the molecular targets affected by single vs. double knockdown, gene expression was analyzed in control, Sep15-, TR1- and TR1/Sep15-knockdown CT26 cells using microarrays. Our analysis revealed that the expression of several genes, e.g., serologically defined colon cancer antigen 1 *(Sdccag1*), were decreased significantly in both single-knockdown but not in double-knockdown cells. However, the mRNA expression of guanylate binding proteins, Ifi44, and other inflammation-related genes, which were highly increased in shSep15 knockdown cells, were no longer up-regulated in TR1/Sep15 cells. Furthermore, shTR1 cells, which also displayed a reversal in cancer phenotype, displayed fewer inflammation-related gene changes, but instead showed decreases in genes such as *Ucc1* (upregulated in colorectal cancer gene 1). The results suggest that a) the similar reversal in cancer phenotype observed by the independent down-regulation of either Sep15 or TR1 appear to result through different molecular mechanisms, b) double-knockdown of both genes no longer results in changes of the cancer phenotype of mouse colon carcinoma cells, indicating possible competing/interfering pathways of regulation of Sep15 and TR1. Funded by NCI Intramural support, the Cancer Prevention Fellowship Program, Towson University and NIH grants.