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Molecular mechanisms by which selenoproteins affect cancer risk and progression

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1. Introduction

Selenoproteins represent a family of peptides that contain the amino acid selenocysteine inserted co-translationally in response to an in-frame UGA codon. This class of proteins is to be distinguished from proteins in which selenium is present due to the non-specific substitution for sulfur in sulfur-containing amino acids and proteins that covalently bind a selenium atom. This later category includes the selenium binding protein-1 (SBP1) whose reduced expression is associated with a less favorable outcome of colon cancer [1,2].

There is considerable interest in determining whether the chemopreventive properties of selenium might be mediated through its consequential effects on selenoprotein function. The ability of selenium, provided as a dietary supplement to experimental animals, to reduce carcinogen induced and spontaneous cancer incidence in these model systems has been documented for over 20 years and the amassed literature includes the demonstration of an anti-cancer effect for selenium in most organs and against a particularly wide range of carcinogens [3]. In humans, there has been significant effort in assessing whether there is a relationship between dietary selenium intake and cancer risk. While the results have generally been inconsistent, a meta-analysis of sixteen studies indicated a pooled relative risk of 0.72 (95% C.I. 0.61-0.84) when cohort studies were assessed and 0.74 (95% C.I. 0.39-1.39) for case-control studies [4]. Similar results were reported the following year analyzing 20 epidemiological studies and separately quantifying selenium levels is serum, plasma and toenails [5]. Using data obtained from 3 different randomized trials assessing the effects of dietary interventions in reducing the risk of secondary colorectal adenomas, it was determined that those in the highest quartile of selenium status, determined from blood samples, were significantly less likely to develop a new adenoma as compared to those in the lowest quartile with an odds ratio of 0.66 (95% C.I. 0.5-0.87) [6]. A subsequent study indicated a statistically significant association between serum selenium levels and advanced colorectal adenoma only when recent smokers were considered with an odds ratio of 0.53 (95% C.I. 0.27-1.01) when the lowest tertile of serum selenium was compared to the highest [7]. Given the existence of selenium–containing proteins, reports such as these have fueled the search for evidence that individual selenoproteins may be the effectors of the benefits of higher selenium status.

Many studies have reported changes in selenoprotein levels in tumors as compared to corresponding normal tissues. However, it is difficult to know which of these differences are causative factors in the development of the cancers and which are due to the acquisition of the

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transformed state that is typically associated with deregulation of a large number of genes. Therefore, this review will focus on those selenoproteins for which there is human genetic data implicating those proteins in cancer risk or progression, and the data in support of possible mechanisms of action will be discussed.

2. An animal model to investigate the role of selenoproteins in cancer

Although there are a large number of reports demonstrating that various forms of selenium were capable of reducing cancers in rodents, these studies cannot distinguish whether the observed reduction in tumors is due to an effect on small, non-protein forms of selenium or changes in the levels or activity of any of the 24 selenoproteins in these species [8]. There are a significant number of *in vivo* and *in vitro* studies, typically using relatively high levels of selenium well above that likely to increase the levels of selenoproteins, showing anti-tumor, anti-proliferative and pro-apoptotic effects of selenium, and these have recently been reviewed [9]. One transgenic mouse, referred to as the i^6A^-, offered the opportunity to investigate the effects of reducing selenoprotein levels on carcinogenesis while maintaining selenium intake.

2.1 The i^6A^- mouse

The ability for eukaryotic cells to recognize certain in-frame UGA codons as the triplet for selenocysteine requires the selenocysteine-encoding mRNA to contain a recognition sequence in its 3′-untranslated region called a SECIS element, and a host of translation factors dedicated for this particular task [10]. Among these factors is the selenocysteine tRNA ($tRNA^{Ser[Sec]}$) that is initially aminoacylated with serine by a seryl aminoacyl synthetase, the serine is phosphorylated and then converted to selenocysteine. The charged $tRNA^{Ser[Sec]}$ then serves as the adaptor molecule, along with other dedicated translation factors, to appropriately insert the amino acid to the elongating selenoprotein peptide. A derivative $tRNA^{Ser[Sec]}$ was generated by *in vitro* mutagenesis, resulting in the removal of an adenosine and replacing it with a guanosine, a substitution that prevents the conversion of the adenosine to the modified nucleotide N^6-isopentyladenosine [11]. The i^6A^- transgenic mice expressing this mutated tRNA are without overt phenotype, although the levels of most of the selenoproteins are reduced [12]. The development of this model provided an opportunity to independently assess the consequences of selenoprotein deficiency on cancer development.

2.2 Reduced selenoprotein levels enhance carcinogenicity

The i^6A^- mice were assessed for susceptibility to colon cancer by exposure of the animals to azoxymethane (AOM) and subsequent quantification of colonic aberrant crypt foci (ACF) [13]. ACF are a commonly used and established pre-neoplastic biomarker of colon cancer development in rodents, and are likely to similarly represent pre-neoplastic lesions to colon cancer in humans. Colons of i^6A^- mice were shown to express reduced activity of two selenoproteins, GPx-1 and thioredoxin reductase, by 80% and 41%, respectively. AOM-induced ACF's were significantly higher in the colons of i^6A^- mice as compared to isogenic wild type controls. The difference in ACF frequency between the two genotypes was observed at both diets containing adequate and supplemental levels of selenium, 0.1 and 2.0 μg/g diet, respectively. The higher dose of selenium was associated with a decrease in ACF frequency, although this supplemental dose only marginally and non-significantly enhanced the activities of two examined selenoproteins, GPx-1 and thioredoxin reductase, leading the authors to conclude that these data were indicative of both selenoprotein-dependent and –independent mechanisms of colon cancer prevention [13].

Using the same i^6A^- mouse model, it was shown that the reduction in selenoprotein levels resulted in increased development of preneoplastic lesions in the prostate. The approach taken in these studies was to breed i^6A^- mice with C3(1) Tag mice (Tag) that develop prostate cancer

due to the directed expression of the SV40 large and small t antigen oncogenes to that organ [14]. Prostate carcinogenesis was assessed using prostatic intraepithelial neoplasia (PIN) and nuclear atypia as an early biomarker. The levels of GPx-1 were shown to be reduced 5-7 fold in the prostates of bigenic i^6A^-/Tag mice as compared to WT/Tag mice. The bigenic mice demonstrated a statistically significant increase in prostate pathology at the 12, 20, 32 week time points with a positive linear trend for the pathological event, with the effect not being significant at the 42 week time point [14].

While neither of the studies described above can discriminate among which of the selenoproteins were responsible for observed enhanced development of cancer-associated pathology, and nor do they offer many clues in the mechanism by which this occurred, they do provide solid evidence that selenoprotein levels can influence carcinogenesis. Possible targets of selenoproteins relevant to growth control include the stress response signaling kinase Akt and the ribosomal protein S6 kinase (p70S6k) as changes in the expression of both of these molecules were reported in muscle of i^6A^- mice [15]. It has also been shown that the levels of two tumor suppressor genes were altered in mammary tissue in which selenoprotein synthesis was inhibited by the selective deletion of the selenocysteine tRNA in that tissue [16]. Brca1 levels were reduced approximately 3-fold and p53 levels were enhanced approximately 4-fold in the mammary epithelium of these mice. The attenuation of Brca1 may be particularly significant since selenium has been shown to complement the defect in DNA damage repair in lymphocytes obtained from women who contained only one functional *Brca1* gene [17] and the ability of selenium to protect against DNA damage *in vitro* was shown to also require Brca1 [18].

3.1 Selenoprotein P

Selenoprotein P (SePP) is the major selenoprotein in plasma, being responsible for more than 50% of the selenium found. It is unique among selenoproteins as it contains multiple UGA-encoded selenocysteines, ranging from 10-17 per SePP peptide, with the human SePP containing 10 selenocysteines. Screening for differences in the levels of selenoproteins between colorectal adenomas and adjacent tissues from 11 patients, it was reported that SePP mRNA and protein levels were reduced in the adenoma tissue [19]. Subsequently it was similarly shown that SePP levels were reduced in colon cancers as compared from apparently normal tissue from the same individuals. Significantly reduced levels of SePP correlated with histological staging with the higher stage III and IV lesions having less SePP than lower stage lesions (stage I and II) [20]. Reduced levels of SePP were also reported in tumor tissues in paired samples as compared to non-malignant tissue in a study of 33 patients [21]. Reduced levels of expression of SePP were also implicated in prostate carcinogenesis by a comprehensive analysis examining human tissues, mouse tumors and established prostate cell lines [22]. While these studies indicate that reduced levels of SePP are associated with cancer development and perhaps progression, a nested case-control analysis provided direct evidence that reduced SePP levels were associate with cancer risk [23]. This study involved 400 cancer cases with two controls per case, and the odds ratio between the lowest quintile for SePP levels vs. the highest was 5.2 for the trend with a $p < 0.05$. This trend was significant for both cancers of the respiratory track (odds ratio = 6.0) and the digestive track (odds ratio = 3.4).

Further direct evidence for a role for SePP in cancer risk came from studies investigating whether genetic variations within the SePP gene are associated with increased susceptibility to cancer. Two SePP polymorphisms have been shown to have functional consequences [24]. The first of these is a G/A polymorphism that results in either a alanine or threonine at position 234 of the corresponding protein and had been previously reported [25]. The second variation was also a G/A polymorphism that resides in the 3′ untranslated part of the gene. By examining SePP levels in 75 subjects both prior to and after receiving 100 μg selenium in the form of

sodium selenite as part of the SELGEN study, it was determined that the position 234 single nucleotide polymorphism (SNP) was associated with pre-supplementation levels of SePP and the SNP in the 3′- untranslated part of the gene was associated with post-supplementation SePP levels. The authors of this report speculated that the coding SNP might effect protein stability or post-transcriptional modification, while the SNP in the 3′-UTR might regulate translation of SePP in response to available selenium levels [24]. Additional variations and SNPs in the SePP gene of unknown significance, including a complicated variable repeat located in the promoter and coding SNP at position 19 have been reported [26-28].

An association between SePP polymorphisms and colorectal adenomas was reported in a study comparing 772 cases with left-sided adenomas and 777 matched controls that were randomly selected from individuals participating in the Lung, Colorectal and Ovarian Cancer Screening Trial [28]. Four SNPs were associated with increased risk of adenoma, one a rare polymorphisms located in the 5′ portion of the gene present in 9 cases but no controls, 2 in the 3′ non-coding region at positions 31,174 and 43,881with odds ratios of 1.48 and 1.53, respectively. Interestingly, a fourth SNP in the 3′-portion of the gene, at position 44,321 was inversely associated with risk of colorectal adenoma, with an odds ratio of 0.73; 95% CI, 0.057-0.92 [28].

The risk of cancer associated with specific alleles can be difficult to detect if the polymorphisms are of low-penetrance or require additional genetic or environmental factors to allow detection. Such was the case for the position 234 SePP polymorphism. Examining DNA obtained from 2,975 cases of prostate cancer to 1,896 controls, no association between the nucleotide at the polymorphic position and prostate cancer was observed [29]. However, when considered along with another polymorphism associated with cancer risk, that resulting in an alanine at position 16 of the gene for the anti-oxidant protein MnSOD (SOD2), there was a 43% greater risk of prostate cancer compares to men with a valine at the same position of MnSOD (OR = 1.43, 95% CI, 1.17 – 1.76; p=0.0005). There was no risk associated with the valine-encoding *MnSOD* allele as compared to men with the alternative allele that coded for threonine at that position. The risk of prostate cancer for the *ala234* SePP homozygotes rose almost 2-fold for those men with a history of smoking [29]. These results can be explained by the fact the *ala*16 polymorphism, which resides in the mitochondrial leader sequence, results in enhanced transport into that organelle and greater enzyme activity [30]. The enhanced dismutation of superoxide yields hydrogen peroxide, which can be further reduced to water by the enzymatic activity of anti-oxidant proteins such as catalase or the selenium-dependent glutathione peroxidase (GPx-1). The elevated oxidative stress generated by enhanced MnSOD activity is associated with higher cancer risk if the individual is low in anti-oxidants such as selenium [31] or, as discussed below, polymorphisms in *GPx-1* that alter that enzymes activity [32].

The major functions of SePP are consistent with the human clinical data presented above [33]. Animal studies in which SePP was knocked out have established its role as a selenium carrier protein from the liver to peripheral tissues [34,35]. Failure to regional supply adequate levels of selenium could result in the reduction of pools of selenium required for the generation of anti-carcinogenic non-protein selenium metabolites (reviewed in [36]). Effects of SePP on tissue selenium levels could similarly influence the synthesis of other selenium containing proteins, several of which are anti-oxidants and are discussed in greater detail below. For example, it was demonstrated that SePP prevented tert-butylhydroperoxide mediated oxidative damage in an endothelial human cell line [37] or human astrocytes [38] via the induction of the GPx-1 selenoprotein. Similarly, it was shown by selectively removing the selenocysteine tRNA in livers of transgenic mice, resulting in the dramatic reduction of SePP levels, there was a consequential reduction in the levels of another selenoprotein, GPx-1, in both serum and the kidney [39].

In addition to effects on selenium dependent proteins, SePP has also been directly implicated as an anti-oxidant protein. Specifically depleting and enhancing SePP from human plasma resulted in enhanced sensitivity and resistance, respectively, to oxidation and nitration reactions following exposure to peroxynitrate [40]. Purified SePP was shown to function as a phopholipid hydroperoxide glutathione peroxidase that was capable of using reducing equivalents provided by a variety of thiols [41]. It therefore seems likely that the direct and indirect effect of SePP on anti-oxidant defenses could account for its role in cancer risk, and in particular, explain the interaction with *MnSOD* at-risk polymorphisms in determining susceptibility to cancer [29].

4. Glutathione peroxidases

4.1 Glutathione peroxidase-1

GPx-1 is found in the cytosol and mitochondria, and has the primary function of detoxifying hydroxyperoxides, thereby reducing oxidative stress that could result in DNA damage that is potentially mutagenic. It is a ubiquitously expressed enzyme containing four subunits, each of which contain selenium as the amino acid selenocysteine. GPx-1 is polymorphic at several positions [26] and several specific GPx-1 alleles have been associated with cancer risk (Table 1). The GPx-1 gene is polymorphic at codon 198, resulting in either a proline (Pro) or leucine (Leu) at the corresponding position of the encoded peptide. By examining the frequency of *GPx-1* genotypes in normal tissues and tumor samples, we have shown significant differences for cancers of the head and neck, breast and colon [42-44] and numerous case controlled studies have indicated that a leucine at position 198 has been associated with increased risk for several cancer types [45-50]. For example, a case controlled study examining the association between polymorphisms in *GPx-1* and bladder cancer indicated that the *Pro/Leu* genotype was associated with increased risk of bladder cancer compared to *Pro/Pro* genotype and that having *Pro/Leu* genotype was associated with more advanced tumor stage [47]. In the case of lung cancer, individuals with the *Pro/Leu* or *Leu/Leu* genotype were at higher risk for lung cancer [50]. Patients with lung cancer had higher levels of urinary 8-OH-dG levels, a common pro-mutagenic DNA lesion, compared to controls, and urinary 8-OH-dG levels were higher in those patients with the at risk genotype compared to the *Pro/Pro* genotype [50]. Individuals with alcoholic liver cirrhosis with the *Pro/Pro* genotype were shown to be less likely to develop hepatocellular carcinoma than patients expressing the *Leu* allele. This increased risk of hepatocellular carcinoma was also seen in conjunction with an at-risk *Ala/Val* manganese superoxide dismutase (*MnSOD*) gene polymorphisms [49]. This association between the *Leu* allele and increased cancer risk has not be seen in several studies [28,51-54] and there may be many possible reasons for this, including interactions with other genetic and environmental risk factors, as noted above for MnSOD. This is exemplified by the negative study reported by Cox for breast cancer [52] being followed by a report from the same group indicating a significant association between the at-risk alleles for both GPx-1 and MnSOD and breast cancer risk [32]. In contrast to these results, others have described data indicating that the *Pro* allele may under certain circumstances offer protection instead of risk [55-57]

In addition to the codon 198 polymorphism, a trinucleotide repeat variation resulting in either five (ALA5), six (ALA6) or seven (ALA7) alanine repeats in the amino terminus of the GPx-1 protein has been reported [58]. The number of repeats is associated with cancer of several types but there is no apparent pattern for which allele is associated with risk, as seen for the codon 198 variation [59-61].

It would be anticipated that genetic variations that increase disease risk would alter the function of the corresponding protein. In the case of the codon 198 polymorphism, erythrocyte GPx activity levels were shown to be lower in patients expressing the *Leu* allele [45]. Using an *in vitro* approach, MCF-7 breast cancer cells that express marginal endogenous GPx activity were tranfected with constructs that express either the *GPx-1 Pro* or *Leu* genotypes and the assayed

enzyme activity showed differences in response to selenium supplementation, with the *GPx-1Leu* containing enzyme being least responsive to Se induction [42].

Loss of heterozygosity at the GPx-1 locus is a common event in cancer development—DNA damage leading to genetic alterations can occur in the form of chromosomal breaks leading to allelic loss of protective genes such as tumor suppressor genes and others involved in regulating the cell cycle and DNA repair. Allelic loss in tumor tissue can be detected when only a single allele is apparent in DNA derived from the tumor while that individual is germline heterozygous. Allelic loss of one of two GPx-1 alleles on chromosome 3p is a common event in the development of several types of cancer, including that of the lung, breast and cancers of the head and neck [42,43,58]. Loss of heterozygosity (LOH) at one or more markers on chromosome 3p, where the GPx gene is located, was also seen in 40% of lung tumors [62]. Tumors with chromosome 3p LOH have reduced glutathione peroxidase activity compared to tumors without 3p LOH [62]. LOH at the GPx-1 locus was reported to occur in approximately 25% of colon cancers examined by laser capture microdissection as compared to the germline DNA from the same patients [44]. Interestingly, LOH at the GPx-1 locus may be an early event in cancer development as suggested by results indicating LOH in histologically normal tissue surrounding head and neck tumors [43]. Allelic loss of one of two GPx-1 alleles may result in reduced enzyme levels, a phenomenon known as haploinsufficiency, or it may unmask a recessive mutation in the remaining allele.

Previous work showed that supplementation of the culture media of CHO cells with small amounts of selenium (30 nM) in the form of sodium selenite increased GPx-1 activity significantly and also reduced the mutation frequency at the *hprt* locus following exposure to 8 Gy of X-rays in these same cells [63]. Other studies have more directly established the benefits of enhanced GPx-1 levels in the protection against DNA damage. Both selenium supplementation to the media of MCF-7 human breast carcinoma cells as well as GPx-1 over-expression achieved due to the expression of a GPx-1 expression construct independently conferred protection of these cells against UV-induced DNA damage as measured by the formation of micronuclei in irradiated cells [18]. Conversely, reduction of GPx-1 activity using siRNA approaches elevated the levels of UV-induced DNA damage in LNCap human prostate cancer cells [64].

How GPx-1 levels might protect cells from DNA damage remains unknown. The antioxidant activity of the protein is anticipated to directly eliminate reactive oxygen species that are potentially mutagenic. It was first shown that over-expression of GPx-1 in B cells could inhibit apoptosis in 1994 [65] and GPx-1 activity has also been shown to influence signaling pathways that are associated with stress response to DNA damage. Over-expression of GPx-1 in human breast-derived cell lines has been shown to alter akt phosphorylation and gadd45 protein levels [66]. Gadd45 is a protein that is induced following DNA damage and its expression has been shown to regulate the G2/M cell cycle arrest, DNA repair, genome instability and apoptosis [67-70] The tumor suppressor protein p53 can serve as a transcriptional activator of brca1, a gene commonly mutated in cancer, and brca1 can serve as a transcriptional activator of gadd45 [71]. Both p53 and brca1 have recently been directly shown to be involved in the mechanism by which selenium reduces DNA damage [72]. Given these results, it is interesting that selenium was shown to be ineffective in reducing DNA damage in a brca1 null background, but not in the corresponding wild type cells [18].

4.2 Glutathione peroxidase 3

Among the glutathione peroxidases, GPx-3 is a secreted glycoprotein present in the plasma [73]. Although the proximal tubule of the kidney was shown to be a major source of GPx-3 [74], it is produced by a variety of other tissues as well [75,76]. Evidence for a role of GPx-3

in prostate cancer was obtained as it emerged as a gene which was frequently CpG methylated in primary prostate cancer samples and cell lines [77]. Methylation of CpG islands in the promoter regions typically results in transcriptional silencing, and cancer-specific methylation often identifies beneficial genes whose down-regulation contributed to cancer development [78]. In this study, *GPx-3* was CpG methylated in 38 of 41 (93%) of prostate cancer samples, as well as several human prostate cancer cell lines and a bladder cancer cell line [77]. Of interest was the observation that *GPx-3* was also methylated in 2/9 samples of patients with benign prostate hyperplasia, indicating that the down-regulation of *GPx-3* occurs in different types of prostate pathology. Similar results were subsequently reported indicating that *GPx-3* was methylated in 90% (27/30) of prostate tumor samples and either one or two copies of the *GPx-3* gene were deleted in 39% of the tumor samples analyzed [79]. An inverse association between *GPx-3* expression and tumor grade was also observed.

A direct effect of elevating GPx-3 levels in human prostate cell lines was obtained by transfection of a GPx-3 expression construct into PC-3, DU145 and LNCaP cells and showing a consequential reduction in the ability of transfectants to grow in semi-solid media, a frequently used measure of cellular transformation [79]. PC-3 transfectants over-expressing GPx-3 also grew slower and were less metastatic when injected into immunosuppressed mice. One possible mechanism by which increased GPx-3 could have influenced these biological effects was indicated by the observation of reduced expression of the c-met protoncogene in the PC3 transfectants [79].

In addition to data indicating a role in prostate carcinogenesis, reduced levels of GPx-3 and hypermethylation were also shown to occur frequently in Barrett's esophogous, a premalignant lesion where the normal esophageal epithelium is replaced by intestinalized epthilium with goblet cells [80]. These studies collectively support the likelihood that reduced levels of GPx-3 promote carcinogenesis. At-risk GPx-3 polymorphisms have not been reported to date for cancer, although polymorphisms located in the promoter region of GPx-3 that reduce transcriptional activity have been shown to be associated with increased risk of arterial ischemic stroke [81-83].

4.3. Glutathione peroxidase-4

GPx-4 is another member of the glutathione peroxidase family of selenoproteins that has received considerable attention. It is a ubiquitously expressed anti-oxidant protein that is unique among the glutathione peroxidases in that in resides in the membrane fraction and can reduce and detoxify lipid hydroperoxides, including phospholipid hydroperoxides [84-86]. In addition to its role as an anti-oxidant enzyme, GPx-4 also performs a critical structural function in the mitochondrial capsule of the mitochondria [85]. The results of many studies have established that GPx-4 can protect different kinds of cells from a wide variety of oxidative stresses [87] and this conclusion has been substantiated in transgenic mice that either over-express [88-91] or under-express that protein [92].

The effects of GPx-4 expression on the malignant phenotype have been investigated in cell lines derived from pancreatic cancers, which were shown to have lower GPx-4 protein levels than that seen in normal pancreatic tissue [93]. Ectopic GPx-4 expression in these tumor cell lines resulted in reduced plating efficiency and growth in semi-solid media. When injected into the flanks of nude mice, GPx-4 over-expressing tumor cell lines were also shown to form tumors considerably more slowly than either the parental lines or the same cells transfected with an empty vector [93]. The over-expression of GPx-4 in L929 fibrosarcoma cells inhibited their growth in nude mice, but had no effect on the strongly tumorigenic B16BL6 melanoma cell line although the metastatic potential of these transfected cells was attenuated [94]. These data indicate that reduced GPx-4 levels might be associated with carcinogenesis. One study reported the examination of GPx-4 levels in human tissue samples. By comparing GPx-4 levels

in invasive ductal carcinoma tissue and comparing it to benign breast tissue from the same patients, it was determined that there was an inverse association between expression and tumor grade, with low GPx-4 expression being associated with other clinical markers of poor prognosis [95].

A SNP located in the 3′-untranslated region of the *GPx-4* gene has been described [96]. The polymorphism results in either a C or T at position 718, which is in close proximity to the SECIS element required for the insertion of selenocysteine at the single in-frame UGA codon in the GPx-4 mRNA. Additional information about the functional consequences of the position 718 variation was obtained in a human clinical study in which individuals were genotyped at the *GPx-4* locus, provided a selenium supplement and both GPx-4 levels and activity were assessed [97]. Individuals with the *CC* genotype showed an increase in the levels of lymphocyte GPx-1 and plasma GPx-3 levels when provided the selenium supplement, while individuals that were homozygous for the *TT* genotype did not exhibit the same effect. Upon withdrawal of the selenium supplement, GPx-4 levels declined only in *TT* homozygotes and not those homozygous for the CC allele. In vitro studies also revealed that the C containing *GPx-4* 3′-UTR was more efficient in binding protein then the same sequences containing a T at that position [97]. Using a specialized reporter construct designed to quantify the efficiency with which the SECIS element could promote selenocysteine insertion during translation, the functional role of the position 718 polymorphism was experimentally established by showing that the C-containing GPx-4 SECIS element was more efficient than that containing a T, results consistent with the previously reported protein binding capabilities of this RNA element [98].

Clinical data implicating GPx-4 in cancer development was obtained when the frequency of the 718 polymorphism was determined from germline DNA obtained from 252 patients with adenocarcinoma, 107 individuals with adenomatous polyps and 187 cancer-free controls [98]. Allele frequencies were statistically the same between those in the control group and those with polyps, while the *CC* genotype was represented to a significantly higher degree in the cohort with diagnosed adenocarcinoma of the colon. Since the samples obtained for genotyping were blood and not tissues, these data indicate that the *C* genotype may represent an at-risk allele of *GPx-4*. This study clearly provides the impetus to perform larger case-control studies to establish whether this is in fact the case.

In vitro and animal experiments, as well as human genetics have now implicated reduced levels of GPx-4 with increased risk of cancer. As an anti-oxidant enzyme located in the membrane, GPx-4 can play a role in the protection of biomolecules from oxidative damage. In addition to the classical anti-oxidant role, there is considerable evidence that this enzyme can influence a broad range of intracellular signaling pathways, including those that involve NF-kB, the products of lipoxygeneases and cycloxygenases (extensively reviewed in [84,87,99]) and effects on these pathways are likely to influence the cellular apoptotic response to external stimuli. Transgenic mice engineered to over-express GPx-4 were protected against diquat and *t*-butylhydroperoxide mediated oxidative stress [88], and consistent with this observation, the reduction in GPx-4 levels in cells derived from hemizygous GPx-4 mice ($GPx4^{+/-}$) were sensitized to oxidative stresses [92,100]. Surprisingly and contrary to expectations, $GPx4^{+/-}$ mice exhibited an extension of life-span as compared to wild type mice, and the authors of this latter study suggested that the benefits obtained by reduced GPx-4 levels were a direct result of increased apoptosis in target tissue that would have eventually yielded life-threatening pathologies [101]. These examples are cited to emphasize the complexity of possible consequences of alteration in these signaling molecules and stress the need for additional work to establish a role of GPx-4 in cancer development and to understand the mechanisms involved.

5. Sep-15

Sep 15 is a 15 kDa selenoprotein shown to be a mammalian selenoprotein located in the endoplasmic reticulum and whose gene resides on human chromosome 1 [102]. Its function includes the formation of disulfide bonds that assures proper protein folding, a role facilitated through its interaction UDP-glucose:glycoprotein glucosyltransferase [103,104]. An examination of the dbEST database indicated that the gene for Sep15 was polymorphic with a C/T variation at position 811 and a G/A polymorphism at position 1125, both positions being included in the predicted SECIS element required for selenocysteine insertion during translation [102]. The examination of over 700 DNA samples indicated the existence of only two haplotypes, with either a T at position 811 and an A at 1125, or a C at 811 and a G at 1125, with the *TA* haplotype being relatively rare: homozygotes representing only 7% for Caucasians but considerably higher, 31%, in African Americans [105].

The examination of *Sep15* allele frequencies in DNA obtained from human tumors indicated that the distribution was significantly different than that seen in DNAs obtained from cancer-free individuals with fewer heterozygotes among DNAs obtained from either breast tumors or cancers of the head and neck [105]. The experimental design of this study, comparing the allele frequencies of archived tumor samples vs. data obtained from cancer-free and ethnicity matched controls, did not permit evaluation as to whether there were certain *Sep15* alleles that contributed to cancer risk, or whether there was LOH as the tumors developed. However, one example of LOH was documented when lymphocyte DNA was compared to head and neck tumor DNA, both samples obtained from the same individual [105]. LOH usually involves the loss of large expanses of genomic DNA, and a follow-up study examining highly polymorphic microsatellite markers spanning the region of human chromosome 1 that include the *Sep15* locus indicated that only that marker most tightly linked to *Sep15* was consistently lost in breast cancer samples, consistent with the idea that the loss o*f Sep15* of another tightly linked gene was contributing to cancer development [106].

A role for Sep15 in cancer development was also indicated for different types of lung cancer. Malignant mesothelioma (MM) is an aggressive cancer of the mesothelial lining of the lung whose development is often associated with asbestos exposure. Expression of Sep15 is reduced in cell lines derived from tumors of this type compared to the corresponding normal tissue, and the reduced frequency of heterozygotes among MM cell lines compared to that seen in the cancer-free population was interpreted to being indicative of frequent *Sep15* LOH [107]. Recently, an association between *Sep15* genotype and small-cell or non-small cell lung cancer as a function of selenium status revealed that there was an increased risk of lung cancer in those individuals with an *AT* haplotype and low selenium status [108].

Using reporter constructs designed to investigate the efficiency of SECIS element function, the consequences of *Sep15* 3′-UTR polymorphisms were revealed with the SECIS element containing the *AT* haplotype being less responsive to the supplementation of the culture media with low levels of selenium [105,109]. Selenium-mediated apoptosis of MM cell lines was investigated, and it was determined that 1) cell lines with the *AT* haplotype were less susceptible to selenium-mediated growth inhibition and apoptosis, 2) selenium-induced apoptosis was enhanced by the introduction of *GC* variants of Sep15 but the not the *AT* variant and 3) the reduced levels of Sep15 achieved using siRNA resulted in increased resistance to selenium toxicity [107]. Collectively, these studies tend to support a functional consequence of the *Sep15* 3-UTR polymorphism with the *AT* haplotype resulting in lower Sep15 protein levels and a reduced susceptibility to apoptosis that may sensitize premalignant cells to removal by apoptosis. This effect may be mediated through the ability of Sep15 to assist in the correct folding of cellular proteins.

6. Other selenoproteins

This review has focused on those selenoproteins for which there is accumulating human genetic evidence indicating a possible role in cancer risk of development. These criteria have been mostly restricted to either epidemiological data indicating at-risk alleles or the frequent LOH of selenoprotein genes. In addition to the consideration of the selenoproteins discussed above, several other are likely to play important roles in canter etiology. Among these is another member of the glutathione peroxidase family of selenoproteins, GPx-2, whose expression is essentially limited to epithelial cells. Mice in which both *GPx-1* and *GPx-2* have been deleted show a dramatic increase in susceptibility to gastrointestinal cancers, but not when the experimental animals were maintained under germ-free conditions, a phenomenon attributed to effects of the depletion of these GPx's on intestinal inflammation [110]. These double knockout mice are also more susceptible to the accumulation of mutations in the cells of the intestine [111]. GPx-2 knockout mice are also more susceptible to the induction of squamous cell carcinoma of the skin following exposure to ultraviolet light [112]. Readers are directed to an excellent review of the likely mechanisms by which GPx-2 deficiency promotes intestinal cancer [113].

Another selenoprotein whose activity is likely to influence cancer development is thioredoxin reductase (TR1). TR1 reduces the active site of its target protein, thioredoxin, and together have been implicated in numerous processes associated with carcinogenesis (for reviews, see [114,115]). *In vitro* studies assaying transformation-related properties of tumor cells in which TR1 are knocked down have indicated attenuation of several of these characteristics, implicating elevated levels of this selenoprotein in carcinogenesis [116,117]. There has been a single report of an association between a C/G polymorphism in the *TR1* gene and cancer, indicating a very significant correlation between a nucleotide variation (IVS1-181) and the risk of advanced colorectal adenoma [28]. However, the authors point out that this polymorphism is located only 30 nucleotides 3′ from the start codon and a putative transcription factor binding site for another gene, E1A-like inhibitor of differentiation 3 (*EID3*) which may be accounting for the adenoma risk [28].

7. General comments and conclusions

Human genetics have uncovered a class of selenoproteins that are very likely to be important factors in the development of cancer. Germline polymorphisms in the genes for these selenoproteins that are associated with the risk of cancer are strong indications that the consequential effects on either gene regulation or protein function can contribute to increased probability that the series of events required for malignant conversion will in fact occur. Similarly, transcriptional silencing by epigenetic mechanisms and LOH in cancerous tissues also indicates that these events likewise contribute to the clonal evolution of tumor cells. While there are several different mechanisms by which selenoproteins might reduce cancer risk, most of the proteins discussed above have demonstrated anti-oxidant activity which might reduce reactive oxygen species that might either damage biomolecules or induce signaling pathways that inappropriately promote cellular proliferation. It is also apparent that consideration of the roles for each of these genes in carcinogenesis should be considered in conjunction with their effects on other selenoproteins. Less delivery of selenium to peripheral organs, as would occur in cases of reduced SePP, will reduce the activity of proteins that require selenium for their synthesis and function. The interactions among selenium availability, polymorphic genes for selenoproteins and alleles for MnSOD also indicate the complexity that will be faced as future studies attempt to understand the impact of genetic variation within selenoprotein genes and cancer etiology.

The genes for these selenoproteins are therefore candidate “tumor suppressor genes” that are potential targets for interventions to either prevent or slow the development of cancer. One such approach might include selenium supplementation if it were established that protective activities of these selenoproteins could be enhanced in the target tissues at non-toxic doses and forms of this element. It is noteworthy that the large SELECT trial designed to investigate the efficacy of selenium supplementation, alone and in concert with vitamin E, was recently terminated early due to lack of evidence of efficacy and data indicating that selenium supplementation may actually have adverse effects [118]. In light of the genetic data presented herein, it remains possible that the benefits of selenium supplementation may not become apparent until participants in trials are stratified by genetics to identify those individuals whose risk of cancer can be reduced by this approach.

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Table 1

Human studies indicating an association between the *GPx-1 Pro198Leu* polymorphism and cancer risk

Cancer type	Population	At-risk allele	References
Lung	Finland	Leu	Ratnasinghe et al., 2000 [46]
Lung	USA	Leu	Yang et al., 2004 [57]
Lung	Korean	Leu	Lee et al., 2006 [50]
Breast	Denmark	Leu	Raven-Haren et al., 2006 [45]
Breast	USA	Leu	Cox et al., 2006 [32]
Bladder	Japan	Leu	Ichimura et al., 2004 [47]
Bladder recurrence	USA	Leu	Zhao et al., 2005 [119]
Liver	France	Leu	Sutton et al., 2006 [49]
Lymphoma	UK/USA	Leu	Lightfoot et al., 2006 [48]
Lung	USA	Pro	Yang et al., 2004 [57]
Lung	Denmark	Pro	Raaschou-Nielsen et al., 2007 [56]
Prostate	Macedonia	Pro	Arsova-Sarafinovska et al., 2009 [55]

Table 2

Human studies indicating an association between polymorphisms within selenoprotein genes and cancer risk

Selenoprotein	Chromosome position	Allelic variation	Location within gene[ab]	Associated cancer types
SePP	5q31	G/A (rs3877899)	CS	Prostate [29]
		G/A (rs7579)	3′NC	[24][c]
		C/G	5′NC	Colorectal adenoma [28]
		A/G (rs12055266)	3′NC	Colorectal adenoma [28]
		A/G (rs3797310)	3′NC	Colorectal adenoma [28]
		C/T (rs2972994)	3′NC	Colorectal adenoma [28]
GPx-1	3p21.3	C/T (rs1050450)	CS	Lung [46,50,56,57], breast [32,45], bladder [47,119], liver [49], lymphoma [48], and prostate [55].
		GCG repeats	CS	Breast [59], prostate [61], and head and neck [60]
GPx-4	19p13.3	C/T (rs713041)	3′NC	Colorectal cancer [98], [97][c]
Sep15	1p31	C/T	3′NC	Lung [108]
		G/A	3′NC	Lung [108]

[a] CS; coding sequence.

[b] NC; non-coding sequence.

[c] Polymorphisms associated with differential response to selenium.