Chapter 37
Interplay of Selenoproteins and Different Antioxidant Systems in Various Cancers

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Abstract Malignant tumors are known to require robust antioxidant systems to sustain their rapidly dividing cells and protect them from oxidative damage. The dietary trace mineral selenium, through its incorporation into selenoproteins such as thioredoxin reductase 1 (TXNRD1), glutathione peroxidase (GPX) 2 and the 15 kDa selenoprotein (SEP15), has been shown to play important roles in redox-regulation. Given that the functions of these selenoenzymes protect both normal and malignant cells from oxidative stress, these very same redox-regulatory processes are thought to result in both anti- and pro-tumorigenic effects at a tissue-specific and cellular level; thus, these selenoproteins are often referred to as having a "Dr. Jekyll and Mr. Hyde personality". Herein, we summarize the main findings with emphasis on TXNRD1 and SEP15, and their roles in the regulation of specific studies of lung, liver and colon cancers to illustrate the differences in the antioxidants involved, and the complexities of their interplay with other antioxidants or antioxidant systems. It should be noted that it remains to be established if any of the observed anti- and pro-tumorigenic effects of TXNRD1 and SEP15 are possibly tumor stage or grade-dependent.

Keywords 15 kDa selenoprotein • Carcinogenesis • Glutathione system • Glutathione peroxidase 2 • Liver cancer • Lung cancer • Oxidative stress • ROS • Selenoprotein interplay in cancer • Thioredoxin reductase 1 • Thioredoxin system

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37.1 Introduction

Selenium (Se) has been known for many years to serve as a chemopreventive agent that suppresses various cancers, largely from epidemiological and animal studies [1]. However, more recently, this element has also been shown to play major roles in promoting cancer in the form of selenoproteins ([2–7] and Chaps. 16, 19 and 38). The fact that Se was found to enhance the cancer process made perfect sense, as this element is one of nature's most potent antioxidants, and cancer cells are known to suffer from oxidative stress.

Numerous studies have shown that Se, in the form of thioredoxin reductase 1 (TXNRD1), is enriched in many cancer cells and tumors, and the inhibition of TXNRD1 might provide an avenue for therapy (see [8–14] and Chap. 16). These studies demonstrated that this selenoenzyme plays a role in driving or sustaining cancers. The molecular biology of Se's role in cancer promotion became more clearly defined, in part, when three selenoproteins, TXNRD1, the 15 kDa selenoprotein (SEP15) and glutathione peroxidase (GPX) 2, were shown to exhibit a Dr. Jekyll and Mr. Hyde personality in preventing and promoting malignancy (reviewed in [7, 15–17] and Chaps. 16, 19 and 38). All three of these selenoenzymes appear to take on an anti- or pro-carcinogenic identity depending on cancer type and stage of the cancer.

It should also be noted that GPX4 has, more recently, been shown to be a major player in cancer through the recognition of its role in a newly discovered phenomenon, designated ferroptosis (Chap. 43 and recent review [18]). Ferroptosis is an iron-dependent form of non-apoptotic cell death and GPX4 has been shown to be an essential regulator of ferroptotic cancer cell death.

In normal cells, a complex interplay exists among antioxidant selenoproteins, and/or between antioxidant selenoproteins and other antioxidants in combating reactive oxygen species (ROS) by maintaining a relatively stable equilibrium. Similarly, such complex interactions also exist in cancer cells, but usually at much enhanced levels that differ vastly from those of the corresponding normal cells from which they originated. These complex relationships also differ substantially among different tissues and cancer types.

Liver and lung tissues, and the different cancers that arise from them provide specimens for comparing how they utilize different antioxidants to maintain redox homeostasis in normal tissue, and how the antioxidants change in combating the enhanced ROS in malignancy. These normal and neoplastic liver and lung cells are also discussed herein. In addition, the interplay between two different selenoprotein antioxidants in colon cancer cells, wherein their individual loss reversed the cancer phenotype, but their collective loss restores cancer properties, is also discussed. Initially, however, elevated ROS levels, which are one of the hallmarks of cancer cells [19], and the manner in which cancer cells cope with oxidative stress, permitting them to grow at accelerated rates outdistancing neighboring, normal cells, is addressed. The underlying metabolic reasons governing how these selenoproteins maintain redox homeostasis to keep cells healthy, and how they are enriched or reduced in malignancy have also been described elsewhere in this book (Chaps. 16, 19 and 38).

37.2 ROS in Cancer Progression and Regression

Much attention has been dedicated to the role of ROS in cancer cells, since enhanced redox signaling and oxidant stress have been shown by many investigators to initiate and sustain cancers and, therefore, are considered prime targets in cancer therapy (reviewed in [11, 20–26]). The two major antioxidant systems in mammalian cells are the thioredoxin (TXN) and glutathione (GSH) systems, which numerous researchers have focused on, either individually [11, 27, 28] or collectively [23, 29–31], as constituents to encumber or reverse the malignancy process. One of the principal problems with down-regulating any antioxidant enzyme, or enzymes, or a system, is that the loss of expression of one or more of these components may induce expression or activity of other antioxidants, which can readily combat the enhanced oxidative stress generated by the malignancy, permitting the cancer cell to thrive (see below).

Other studies, however, have shown that ROS can, instead of initiating and promoting cancer, limit tumor growth and metastasis, illustrating further the complexity of ROS and oxidative stress in these processes. Harris et al. [29] demonstrated that the antioxidant GSH and TXN pathways play different roles by synergizing their efforts. The GSH pathway initiates the malignancy, and then the TXN pathway drives cancer progression. If the GSH pathway is inhibited prior to cancer initiation, the malignancy process can be retarded. However, once the cancer is initiated, its progression is then supported by the TXN pathway and inhibiting the GSH pathway no longer will retard tumorigenesis. Interestingly, inhibition of both pathways resulted in "a synergistic cancer cell death in vitro and in vivo" [29].

The requirement of inhibiting both pathways in retarding tumor growth in TXNRD1-deficient, tumor bearing mice was first demonstrated by Conrad and collaborators [30]. The varying roles of ROS and antioxidants in cancer initiation, progression and/or regression, illustrating the complexity of ROS and antioxidants in these processes, were detailed recently [31, 32]. A recent study that does not involve selenoproteins as antioxidants *per se*, should be mentioned as it further emphasizes the complexity of ROS in cancer ([33] and see review [34]). This study surprisingly found that ROS can retard metastasis in melanoma tumors, while only those malignant melanoma cells with enhanced antioxidant proficiency can actually accomplish metastasis.

37.3 Selenoprotein Roles in Normal and Malignant Lung and Liver Tissues

Lung and liver are two diverse tissues that rely on very different antioxidant systems to maintain redox stability. The corresponding cancers arising in both tissues utilize very different antioxidant systems to drive the respective malignancy. We initially examine the antioxidants primarily involving cancer cells and tumors in mice (see Sect. 37.3.1) and then discuss antioxidants primarily involving cancer cells and tumors in humans (see Sect. 37.3.2).

37.3.1 Selenoprotein Roles in Mouse Normal and Malignant Tissues

Selenoproteins participate in different roles in diverse tissues in promoting malignancy. For example, TXNRD1 is known to maintain redox homeostasis in normal hepatocytes, protecting them from oxidative damage and disease [35]. The loss of this selenoenzyme in hepatocytes, however, was found to greatly increase tumor incidence in liver of mice exposed to the liver carcinogen, diethylnitrosamine (DEN) [36]. The tumor increase appeared to be due to activation of NFE2L2, which in turn enhanced the expression of GPX2 and enzymes in the GSH pathway. These enriched enzymes apparently were responsible for providing the oxidative prowess to combat the enhanced ROS in driving the malignancy.

Other studies have examined the role of Se and selenoproteins in mouse hepatocarcinogenesis with varying genetic backgrounds. Mice encoding the hepatocarcinogenic driver genes, TGFα/c-Myc, were treated with DEN and placed on Se-deficient diets that were, or were not, supplemented with different levels of Se [37]. Interestingly, mice maintained on Se-deficient or highly supplemented Se (2.25 ppm Se) diets suppressed hepatocarcinogenesis compared to mice maintained on intermediate levels. The expression of most selenoproteins correlated with tumor formation in mice on the diets containing intermediate (adequate) levels, while mice on deficient and highly supplemented diets induced expression of detoxifying genes, inhibited cell proliferation and exhibited increased apoptosis [37].

An additional study exposed mice to DEN, wherein the mice encoded a mutant $tRNA^{[Ser]Sec}$ transgene, designated $Trsp^{tA37G}$, which produced reduced levels of non-essential, stress-related selenoproteins [38]. Tumor incidence increased in $Trsp^{tA37G}$ mice fed adequate levels of Se, whereas control, wild type mice fed Se-deficient or highly enriched Se diets were protected from tumor formation [38].

Overall, the above studies on tumorigenesis in mouse hepatocytes suggest a complex role of Se in chemically-induced or genetically driven hepatocarcinogenesis, which involve the interaction of selenoproteins, selenocompounds and chemical carcinogens. Furthermore, changes in dietary Se levels and/or selenoprotein expression in these mice may suppress or promote tumor formation, and the cell type and murine genotype also play roles in governing the malignancy process. TXNRD1 appears to play more of a protective role in hepatocytes guarding against tumorigenicity by maintaining redox homeostasis rather than a cancer promoting role. This may be due to TXNRD1-deficiency in hepatocytes being compensated for by induction of other antioxidant enzymes that can then drive tumor formation [36].

Several studies have suggested that tumor formation in mouse lung tissue and cells is highly dependent on TXNRD1 and the loss of this selenoenzyme is anti-tumorigenic. An earlier study clearly demonstrated that tumor formation in mouse lung cancer (LLC1) cells was virtually completely dependent on TXNRD1 [13]. Several of the cancer hallmarks [19] in TXNRD1-sufficient, LLC1 cells were reversed following targeted down-regulation of *Txnrd1*. Furthermore, tumorigenicity of TXNRD1-deficient LLC1 cells injected into the flanks of mice was dramatically

reduced compared to the corresponding TXNRD1-sufficient cells. The slower growing tumors arising from the TXNRD1-deficient cells were subsequently found to have lost the *Txnrd1* targeting vector and had re-expressed this selenoenzyme, demonstrating unequivocally that lung tumorigenesis in mice, at least regarding this cell line, depended on expression of TXNRD1 [13].

37.3.2 Selenoprotein Roles in Human Normal and Malignant Lung and Liver Tissues

Lung cancer is the number one cause of cancer deaths throughout the world and there are three types, non-small cell, small or oat cell, and metastatic. Each of these lung cancer types includes various forms which are considered to be different cancers. Adenocarcinoma (LAC) is the most common of the non-small cell forms representing about 35 % of all types. There are also several types of liver cancers, which comprise the sixth most common cancer and second leading cause of cancer deaths globally. Hepatocellular carcinoma (HCC) is the most prevalent form, representing about 75 % of all known human liver cancers. This chapter primarily examines LAC and HCC.

In a recent study, the development of lung tumorigenesis incidence in mice carrying a lung cancer gene was investigated. However, the resulting tumor size was increased considerably in those mice maintained on diets supplemented with the known antioxidants, N-acetylcysteine or vitamin E, compared to the littermate controls maintained on normal diets [39]. Low Se levels have also been linked to lung cancer development [40, 41], but questions and concerns have been raised whether Se intervention through dietary supplementation should be used as a strategy in lung cancer therapy [40]. It should also be noted that recurrence of non-small cell lung cancer in patients administered a Se supplement or a placebo manifested virtually no differences in cancer recurrence between the two groups that resulted in the trial being stopped early [42]. However, this trial was difficult to evaluate in light of beneficial or detrimental effects of Se in lung cancer due to its early termination [42].

The redox regulatory systems in HCC and LAC has been examined by comparing each respective tumor to its surrounding normal tissue to elucidate the changes that occurred to enrich the antioxidant capacity of the tumor to meet its needs for sustaining the cancer phenotype [43]. Very pronounced differences were observed in the TXN and GSH systems: TXNRD1 levels were elevated in both tumor types, while TXN levels were only slightly increased in HCC, but highly increased in LAC. Peroxiredoxin 1 (PRDX1), an enzyme within the TXN system, was upregulated dramatically in LAC compared to its surrounding normal tissue, and downregulated in HCC. Major differences were also observed in the GSH system between the two tumors and their respective normal tissues. These variations in antioxidants are summarized in Table 37.1. Interestingly, the role of PRDX1 is to protect against oxidative stress by hydroperoxides, such as hydrogen peroxide and

Table 37.1 Summary of changes in levels of redox components examined in tumor and normal surrounding tissues[a]

Antioxidant	Lung[b]	Liver[b]
TXNRD1	↑[c]	↑[c]
TXN	↑[d]	NS[d]
PRDX1	↑	↓
GPX1	NS	↓
GPX2	ND	NS
GPX4	NS	↑
GSR	NS	↓
GCLC	↓	↓
GSS	NS	↓
GLRX	NS	↓
GGT1	↓	↑
GSTA1	ND	↓
SOD1	NS	↓
CAT	NS	↓
G6PD	↑	↑
Ascorbic acid	NS	NS
Uric acid	NS	↓

[a]Data and table adapted from [43]

[b]↑ or ↓ indicate significant increase (↑) or decrease (↓); NS = not significant; ND = not detected

[c]Specific activity of TXNRD was approximately 1.5 times higher in normal lung than liver tissues, but TXNRD1 was reduced approximately by about half in lung tumor compared to liver tumor

[d]The level of TXN was enriched approximately six times in lung tumor compared to normal tissue by western blotting, while liver tumor was only slightly enriched in liver tumor compared to normal tissue

peroxynitrite, and is itself reduced by TXN [27]. This observation supports the proposal that enriched PRDX1 occurring in lung adenocarcinoma is reduced and maintained in the active state by increased TXN levels [43]. Overall, the data suggested that HCC has a much greater dependency on the TXN system and/or the GSH system to drive the malignancy, while LAC appears to depend largely on the TXN system to drive its malignancy. These findings strongly suggest that different therapeutic targeting strategies would be required to slow or reverse HCC or LAC (see also Concluding Remarks).

Upon targeted downregulation, TXNRD1-deficient human lung cancer A549 cells did not manifest reversal of their cancer properties to the same extent that LLC1 mouse cells did; however, the possibility that A549 cells may have retained sufficient TXNRD1 activity following its knockdown to drive the malignancy was considered [44]. Perhaps another possibility should be considered, in that TXNRD1-deficient A549 cells did not manifest reversal of their cancer properties like LLC1 cells because these two cancer lines are likely quite different from each other and may depend overall on different antioxidants to drive the cancer.

37.4 The Interplay Between TXNRD1 and SEP15 in Colon Cancer

Targeted removal of *Sep15* or *Txnrd1* in mouse colon cancer CT26 cells has been shown to result in reversal of several of the cancer properties such as anchorage-dependent and anchorage-independent growth and impaired ability to metastasize ([45] and see Chap. 19). It was anticipated that the simultaneous down-regulation of both these selenoproteins would result in cells more likely exhibiting a phenotype typically associated with normal (non-neoplastic) cells, since such cells were expected to lack the antioxidant ability to combat increased levels of ROS generated in more rapidly growing cells. Remarkably, the anti-cancer effects found in targeting SEP15 or TXNRD1 loss were reversed and the malignancy phenotype recovered when both genes were simultaneously down-regulated [45]. Various other genes were up- or down-regulated differently in SEP15/TXNRD1-deficient cells compared to their individually loss in CT26 cells, which underscored the complexity of these two selenoproteins in their regulatory roles in colon cancer. For example, interferon-γ-regulated guanylate-binding proteins, which are a family of GTPases that are important in providing protective immunity against viral and microbial pathogens, were highly expressed in SEP15-deficient and poorly expressed in TXNRD1-deficient cells. Members of the Wnt/β-catenin signaling pathway were enriched in TXNRD1 and SEP15-deficient CT26 cells. The data suggest that that these two selenoproteins are involved in quite different regulatory pathways in colon cancer cells, but ones that counter each other's regulatory pathways in colon cancer cells; and furthermore, provide new insights into the complexities of how different selenoproteins may interact when they both are under-expressed.

37.5 Concluding Remarks

In this chapter, we have focused largely on specific cancer studies in mice and humans involving selenoproteins to illustrate the differences in the antioxidants involved in different cancers and tumors, and the complexities of their interplay with other antioxidants or systems. There is, of course, a wealth of information on these topics in many other studies far too extensive to cover in a chapter of this size. For reviews on many other such studies, the reader is referred to several excellent reviews [11, 20–24, 26, 28].

Major efforts have been directed in understanding the underlying causes of enhanced antioxidant and/or ROS levels in cancer cells as a means of providing insights into how to slow or impede the cancer process. The reasoning for employing these approaches is to find avenues of inhibiting specific cancer cells and/or tumor growths. The fact that removal of TXNRD1 in hepatocytes enhances the expression of other antioxidants or antioxidant systems, which then drive the malignancy,

demonstrated that focusing on a single antioxidant or even antioxidant system to retard the malignancy will likely not be successful, as has been shown also for other cancers [29, 30]. Whether inhibition of both the GSH and TXN systems would impede a specific liver cancer type, e.g., HHC, remains to be determined. Focusing on reducing or enhancing ROS levels to impede liver cancer may be an alternative and fruitful avenue to pursue for therapy.

Lung cancer, e.g., LAC, appears to be far more dependent on TXNRD1 and the TXN system, as discussed above. Thus, inhibiting lung cancer (e.g., LAC) as a therapy by attacking TXNRD1 and/or the TXN system would seem a much better approach to pursue than with liver cancer (e.g., HHC).

Since the numerous types of human lung and liver cancers must all be considered as individual malignancies with different ROS and antioxidants driving them, specific therapies must be devised for each, and likely for each of the different stages during cancer development. Albeit the tumor study in mice which suggested that the malignancy was initiated by the GSH system and then sustained by TXNRD1 [29] demonstrated the interplay and complexities between the different antioxidants involved, the intricacies are likely far more multifaceted in many other cancers. There is still vast amount of research to be carried out to unravel the underlying molecular mechanisms and their many interactions in finding specific avenues in cancer therapy.

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