Chapter 19
The 15 kDa Selenoprotein: Insights into Its Regulation and Function

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Abstract The 15 kDa selenoprotein (SEP15) is one of more than 25 selenoproteins found in humans. This protein has been proposed to be involved in redox regulation in the endoplasmic reticulum (ER), although the biological function of SEP15 is still not completely understood. It appears to have strong tissue specificity, and has been shown to have a split personality in terms of cancer initiation and promotion. Polymorphisms in the human *SEP15* gene have been linked to increases in several types of cancer. Thus, among the many selenoproteins, SEP15 continues to generate interest due to its potential implications in human health and disease.

Keywords 15 kDa selenoprotein • Oxidoreductase • Polymorphism • Protein folding • SELM homolog • SEP15 • UDP-glucose:glycoprotein glucosyltransferase

19.1 Introduction

SEP15 belongs to the family of oxidoreductases and has been shown to be involved in quality-control of folding of proteins [1]. It is an endoplasmic reticulum (ER)-resident selenoprotein and a homolog of selenoprotein M (SELM), which is also a selenoprotein with redox activity, thought to be involved in the antioxidant response [2]. *SEP15* was first identified and characterized in human tissues by Gladyshev et al. in 1998 [3]. The *SEP15* gene is located on human chromosome 1p31, a location often deleted or mutated in many cancers. The expression of SEP15, like many other selenoproteins, is regulated by the selenium status of the organism [2]. While the expression of essential, housekeeping selenoproteins,

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such as thioredoxin reductase 1 (TXNRD1), is maintained even at very low levels of organismal selenium, the expression of inducible selenoproteins, such as SEP15, are reduced under conditions of low systemic selenium [4].

19.2 SEP15 Structure and Function

The SEP15 protein consists of 162 amino acids, and the Sec UGA codon (see Fig. 19.1a) is found in exon 3 at position 93 [5]. The SECIS element, a selenocysteine insertion sequence of around 60 nucleotides in length that adopts a stem-loop structure, is present in the 3′-untranslated region (UTR) of its mRNA. There appear to be two alternative transcripts for *SEP15*. The longer transcript variant expresses five exons, whereas the second variant lacks an exon in the 3′ coding region [5]. Human SEP15 was originally isolated and characterized using a human T-cell line and mammalian *Sep15* mRNA was found to be expressed in a wide range of tissues [3] with highest expression levels reported to occur in liver, kidney, testes, thyroid and prostate of human, rat and mouse. *Sep15* appears to be highly conserved in nature [6, 7], and has also been detected in various unicellular eukaryotes [8].

Similar to many other selenoproteins, SEP15 belongs to the class of thiol- oxidoreductases [5, 9]. It contains a thioredoxin-like fold [10], with selenocysteine located in the predicted catalytic position, and also contains an ER-targeting signaling

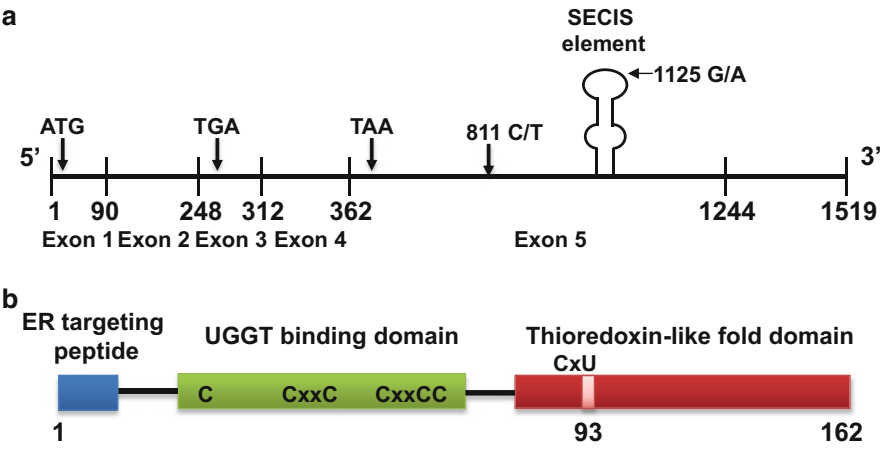

Fig. 19.1 Structure and domain organization of the human 15 kDa selenoprotein (*SEP15*). (**a**) The relative positions of the ATG initiation site, the TGA Sec codon, the TAA termination signal, and the detected polymorphisms (C/T at position 811, and G/A at position 1125 in the SECIS element) are shown. Alternative 3′-end sequences (position 1244 or 1519) are also indicated. (**b**) Protein domains (ER targeting, UGGT binding and thioredoxin-like fold) and location of the Sec codon (position 93) within *SEP15* are shown. The N-terminal domain contains six conserved cysteine residues, four of which form a pair of CxxC motifs important for mediating interaction with UGGT

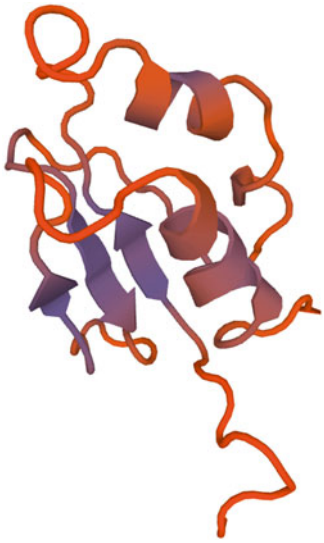

Fig. 19.2 Predicted NMR structure of human SEP15 with α-helices shown in *darker bands*, β-sheets in *purple* (modeled using SWISS-MODEL [47])

peptide in the N-terminal region (Fig. 19.1b) [11]. The structure of its thioredoxin-like domain, as shown by NMR spectroscopy of *Drosophila* SEP15, demonstrated that SEP15 consists of four-stranded β-sheet surrounded by three α-helices, comprising an α/β-fold common for thioredoxin-like oxidoreductases [2]. Human SEP15 is predicted to have a similar structure (Fig. 19.2). Although the thioredoxin domain in SEP15 (CxU) differs from that in thioredoxin and other functionally characterized oxidoreductases, the additional redox-active motifs in a loop between strand β1 and helix α1 further support the suggested oxidoreductase function of SEP15 [2, 12]. The redox potential and thus functional specificity of a protein is determined by the identity of residues in its redox motifs. The redox potential in *Drosophila* SEP15 was found to be −225 mV [9], which lies between known redox potentials of protein disulfide isomerase and the disulfide reductase thioredoxin. Therefore, SEP15 is thought to likely catalyze the reduction or isomerization of disulfide bonds in ER-localized or secretory proteins [9, 12].

Through its Cys-rich domain, SEP15 can form a strong 1:1 complex with UDP-glucose:glycoprotein glucosyltransferase (UGGT), a 150 kDa large ER chaperone enzyme that regulates the calnexin cycle [9], which is responsible for quality control in the ER [13]. Through calnexin, UGGT recognizes misfolded protein domains in the ER of eukaryotic cells and specifically glucosylates these proteins, allowing for another cycle of proper folding [9, 11, 14]. However, unlike its distant homolog, selenoprotein M (SELM), SEP15 lacks an ER retention signal, suggesting that its binding to UGGT is the reason it is retained in the ER [11]. The presence of a thioredoxin domain and its binding to UGGT suggest that SEP15 may contribute to UGGT's function via redox processes and assists in controlling folding or secretion of certain glycoproteins [2]. Thus far, SEP15 has only been found bound to UGGT [11], and the activity of both isoforms of UGGT was shown to be enhanced by the

complex with SEP15 [15, 16]. This suggests that SEP15 may serve as a functional and possibly structural extension of UGGT [17, 18]. Thus, UGGT, together with SEP15, may regulate the calnexin system, as UGGT senses the folding states of glycoproteins [18]. However, SEP15's function in ER quality control has been difficult to validate experimentally.

19.3 Biological Function of *SEP15*

Biological evidence for SEP15's involvement in the regulation of protein folding is observed with the reported prominent nuclear cataract development in eyes of *Sep15* knockout mice early in their life [1], presumably because of accumulation of misfolded proteins in the lens. Additional support for its possible role in protein folding in the lens is also supported by SEP15's co-localized expression with the ER chaperone calnexin and the ER-resident oxidoreductase ERp57, which are both major components of the quality control mechanism in neuronal cells within the ER [14].

Higher versus lower *SEP15* expression in human populations has been linked to altered cancer risks, such as the observation of a decrease of *SEP15* expression in lung cancer patients [19]. Low expression of *SEP15* also has been observed in malignant lung, breast, prostate and liver tissues [20], as well as in cell lines derived from malignant mesothelioma cells compared to normal lung cells [21]. In contrast, a recent global analysis of the hepatic selenoprotein expression showed *SEP15* mRNA to be upregulated in human liver cancer HepG2 and Huh7 cells compared to normal human hepatocytes [22]. Whereas earlier reports in lung, breast, prostate and also liver suggest a role of SEP15 in tumor suppression, these recent observations in liver cancer cell lines and our mouse in vivo studies suggest a role in colon tumor promotion [23].

Interestingly, increased SEP15 expression has been found in response to mild ER stressors such as the antibiotic drugs, tunicamycin and brefeldin A. Additionally, rapid proteasomal degradation of SEP15 has been reported in response to agents inducing a more robust ER stress, such as dithiothreitol [14]. However, decreased levels of SEP15 did not induce ER stress in these studies, indicating that there is a possible compensation mechanism for SEP15 function. Recent studies with a *Sep15* knockout mouse model further implicated its role in redox homeostasis [1]. Although *Sep15* knockout mice appeared normal and did not activate ER stress pathways systemically, parameters of oxidative stress were increased in livers of *Sep15* knockout mice [1]. In contrast, in a murine model of chemically-induced colon carcinogenesis, systemic knockout of *Sep15* decreased development of preneoplastic lesions [24], similar to what has been described in glutathione peroxidase 2 (*Gpx2*) knockout mice [25]. Therefore, even though much of its biological function remains to be elucidated and appears to display aspects of tissue specificity, the importance of SEP15 in health and disease seems supported.

In addition to the development of systemic *Sep15* knockout mice, targeted down-regulation of *Sep15* through RNA interference in mammalian cells in culture has been used to study the biological effects associated with decreased expression of this gene. Based on previous studies, much like TXNRD1, SEP15 may also harbor a split personality in that it has been linked to cancer-preventive [5, 19, 26] and cancer-promoting roles [23, 27], which is discussed in further detail in Chap. 37.

Mouse [27], as well as human colon cancer cells [28], containing shRNA constructs targeting *Sep15* displayed a reversal of the cancer phenotype, including decreased growth abilities compared to control cells. These observations in colon epithelia are in contrast to other published literature, where lowered expression of *SEP15* was reported in many malignant mesothelioma tumor specimens, and increased cell proliferation of mesothelioma cells in vitro [21]. Furthermore, a targeted down-regulation of *Sep15* in mouse Lewis lung carcinoma cells did not affect cell growth [27]. Combined, these results indicated a strong tissue specificity in the response of malignant cells to SEP15 expression.

In an attempt to determine the effect of diminished SEP15 expression in lens epithelium, a study was undertaken in human lens epithelial cells. Targeted down-regulation of *Sep15* alone in these cells did not result in apoptosis, yet it was found that tunicamycin-induced apoptosis and oxidative stress was enhanced in SEP15-deficient cells, while ER stress was not further elevated [29].

Inducible *Sep15* knockdown cell lines were used to examine the role of Sep15 in HeLa/Chang liver cells [30]. Loss of SEP15 was shown to lead to non-apoptotic membrane blebbing and reorganization of cytoskeletal proteins through a RhoA/ROCK/MLCK pathway. *Sep15* knockdown cells were arrested at the G1 phase and the cells also underwent a mild ER stress response. Inhibitors of the RhoA/ROCK pathway in cells with targeted downregulation of *Sep15* recovered the cells' migration and invasive ability, suggesting that SEP15 is involved in cell motility through the organization of cytoskeletal proteins [31].

Interestingly, further investigations of molecular targets affected by the loss of SEP15 in colon epithelium demonstrated a possible link to expression of interferon-γ-regulated inflammatory pathway genes and transcription factors [27, 32]. This finding was further supported by the observation in mice that their serum cytokine levels were affected by SEP15 expression [24]. Possible direct or indirect links between SEP15 expression and pro-inflammatory response continue to be elucidated. Cells regulate function and specific biological activity of cytokines, such as interferons and interleukins, through glycosylation, which may alter their biological activity and overall behavior [33, 34]. Proper glycosylation of cytokines is therefore important for their biological function, and it is possible that SEP15 indirectly affects inflammatory pathways through regulation of glycoproteins, including cytokines.

The interplay or compensatory mechanisms between SEP15 and other selenoproteins, such as TXNRD1, in cancer is less clear. Mouse colon adenocarcinoma CT26 cells have been used to elucidate the roles of both selenoenzymes through targeted downregulation of either *Sep15* or *Txnrd1*, or both [35]. Several of the typical

cancer properties such as cell proliferation and anchorage-independent growth were reversed in these colon cancer cells lacking either SEP15 or TXNRD1, as expected. However, instead of displaying an even stronger reversal of the cancer phenotype as anticipated based on observations with single-knockdowns, the combined down-regulation of SEP15 and TXNRD1 resulted in a recovery of the original malignant phenotype. Interestingly, components of the Wnt/β-catenin signaling pathway, which is frequently dysregulated in non-familial colon cancer, were up-regulated in cells lacking both SEP15 and TXNRD1, suggesting that these two selenoproteins participate in complex, and possibly interfering, specific regulatory pathways in colon cancer [35, 36].

19.4 Human *SEP15* Polymorphisms and Cancer

As with other selenoproteins, single nucleotide polymorphisms (SNP) have been described for *SEP15* (see Chaps. 13 and 29). Among several common SNPs for the *SEP15* gene, two have been reported to have functional consequences [7, 21]. These two SNPs are located in the 3′-untranslated region in the *SEP15* gene (rs5859 and rs5845), and result in C-T substitution at position 811 and a G-A substitution at position 1125, respectively (Fig. 19.1b). Via measurement of reporter gene activity, these SNPs were found to decrease the efficiency of the SECIS element at higher concentrations of selenium [7]. Interestingly, the haplotype with a T at position 811 and an A at 1125 is relatively rare, only occurring in 7 % of Caucasian-Americans, but in about 31 % of African-Americans [7]. The impact of these polymorphisms on the biological activity of SEP15 continue to be elucidated.

A number of epidemiological studies have suggested a relationship between *SEP15* SNPs and cancer risk or mortality, although the evidence remains controversial in many cases. Two previous studies have suggested a possible role of SEP15 in breast cancer. Using DNA obtained from breast tumors as well as from lymphocytes from cancer-free controls, a loss of heterozygosity and a statistically significant difference in allelic distribution for *SEP15* rs5859 in African-American women was reported [7]. A subsequent study supported a possible role of *SEP15* in breast cancer development among African-American women, and demonstrated a significant reduction of heterozygosity for the locus that was most tightly linked to *SEP15* [37]. Recently, the 811C/T (rs5845) *SEP15* polymorphism was further investigated in a Caucasian population of 83 non-familial breast cancer cases and 99 age-matched, healthy controls. The prevalence of the T allele in this Caucasian study population was relatively low, and the genotype variation in breast cancer patients and controls within the 3′-UTR of the *SEP15* gene showed no significant association with breast cancer risk or pathological parameters [38]. Thus, the supporting evidence of a possible role of SEP15 polymorphisms in breast cancer continues to be restricted to women of African-American descent.

For small-cell or non-small-cell lung cancer, an increased risk was found in a Polish population in those individuals with an AA genotype at position 1125 in *SEP15* and low basal selenium status. However, a decreased risk was observed among those with serum selenium levels above 80 ng/mL [19], indicating that those with the AA genotype benefitted from higher serum selenium concentrations. Intriguingly, in those with the GG or GA genotype, a higher selenium status suggested an increase in risk of lung cancer.

The effect of polymorphisms in *SEP15* on prostate and colorectal cancer appears less consistent. In a study conducted in New Zealand, the rs5845 minor T allele was associated with a higher risk for benign prostate disease compared to controls, but a lower risk of developing malignant disease compared to benign disease [39]. In contrast, in the Physician's Health Study, a nested case control study involving 1286 cases and 1267 controls, SNPs in *SEP15* (rs5859, rs479341, rs561104, rs527281, rs1407131) were not significantly associated with risk for prostate cancer [40]. However, prostate cancer-specific mortality was significantly associated with a recessive model of three *SEP15* variants (rs479341, rs1407131, rs561104), and variant rs561104 significantly modified the association of plasma selenium with prostate cancer survival [40]. Similarly, although *SEP15* polymorphism rs561104 in a population-based case-control study of men of European ancestry was initially significantly associated with local stage prostate cancer with an odds ratio of 1.28 for GG versus AA, this association did not remain significant after adjusting for multiple comparisons due to a relatively low sample size [41]. A recent study investigated the impact of genetic variants of *SEP15* and other selenoproteins in a cohort of 722 patients with localized or locally advanced prostate cancer. However, unlike SNPs in *TXNRD2* and selenium binding protein 1 (*SELENBP1*), genetic variants of *SEP15* were not associated with cancer aggressiveness at diagnosis or with plasma selenium levels [42].

No association was found between *SEP15* polymorphisms and colorectal cancer incidence in a case control study in the Czech Republic [43] or in a Korean patient population [20, 44]. However, a significant two-loci interaction between selenoprotein P and *SEP15* variant rs5859 was observed in colon cancer cases from the Czech Republic [43]. Additionally, a gender-specific increased rectal cancer risk in Korean men was associated with the minor alleles for rs5845 (GG-GA), rs5859 (CC-CT) and rs34713741 [20, 44]. Moreover, recent studies in the US also failed to find an association between these SNPs in *SEP15* and colorectal cancer [45]; however, there appeared to be an association between a different *SEP15* polymorphism (rs9433110) and survival after diagnosis with colon and rectal cancer [46].

It appears that the literature regarding the relationship between *SEP15* polymorphisms and cancer remains rather controversial. Gene (mRNA) expression analyses suggest that *SEP15* expression as a function of selenium availability varies among various polymorphic populations, at least in part, due to human polymorphisms in the *SEP15* gene. How these SNPs correlate with SEP15's biological activity, and how SEP15 protein expression and activity may influence cancer risk or mortality in various subpopulations defined by their ethnic/cultural background or otherwise, remains to be validated.

19.5 Concluding Remarks

SEP15 is an ER-resident selenoprotein thought to be involved in quality control of protein folding. Although its pairing with UGGT and the observations of altered inflammatory responses in mice suggests regulatory control over important glycoproteins such as cytokines, its specific biological function remains to be elucidated. A strong tissue specificity and split personality in terms of cancer initiation and promotion has been described for *SEP15*. Polymorphisms in the 3′-UTR lead to differential expression of *SEP15* among populations, and studies suggest a possible relationship to cancer risk. Further research is needed to evaluate the importance of this gene/protein in human health and disease.

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