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Running Title: Serum proteomic profile following selenium-enriched yeast supplementation

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Abstract

Background: Studies demonstrated that supplementation of adult men with selenium-enriched yeast (SY) was protective against prostate cancer (PCa) and also reduced oxidative stress and levels of PSA. Here we determined the effect of SY supplementation on global serum protein expression in healthy men to provide new insights into the mechanism of selenium chemoprevention; such proteins may also serve as biomarkers of disease progression.

Methods: Serum samples from 36 adult men were obtained from our previous SY clinical trial, 9 months after supplementation with either SY (247 μg/d) (n=17) or placebo (non-enriched yeast) (n=19).

Results: Proteomic profiling using 2D-DIGE followed by LC/MS/MS revealed a total of 1496 candidate proteins, of which, 11 were differentially expressed in the SY group as compared to placebo. Eight proteins were up-regulated (clusterin isoform 1 [CLU], transthyretin, α-1B-glycoprotein, transferrin, complement component 4B proprotein, isocitrate dehydrogenase, haptoglobin, keratin 1) and 3 proteins were down-regulated (α-1 antitrypsin [AAT], angiotensin precursor and albumin precursor) by SY. All of the identified proteins were redox-sensitive or involved in regulation of redox status. Since both ATT and CLU have been previously linked to PCa development, their identities were confirmed by 2D Western blot analysis.

Conclusions: We identified AAT and CLU as potential candidate proteins involved in the mechanism of PCa prevention by SY. Collectively, proteins identified in this study may serve as potential new biomarkers for monitoring and comparing responses to selenium-based chemopreventive agents.

Impact: Proteomic analysis of serum may be useful for early detection and monitoring efficacy of chemopreventive agents.
Introduction

Prostate Cancer (PCa) presents a major clinical and public health challenge in the USA. It is the second leading cause of cancer-related deaths in men and second only to lung cancer (1). Men have a 1 in 6 lifetime probability of being diagnosed with PCa. PCa has surpassed heart disease as the top killer of men over the age of 85 years in the USA; 192,000 men were diagnosed with PCa and 27,360 died from this disease in 2009 (1). The incidence and mortality of PCa vary significantly across ethnic groups with African American (AA) men having the highest rates in the world (2). Though the etiology of PCa remains poorly understood, epidemiological studies have revealed a number of risk factors including diet, lifestyle and environmental factors that significantly contribute to the development of this disease (3, 4).

Diet derived-agents including selenium have been shown to have chemopreventive potential against PCa (5). Based on the epidemiologic evidence as well as preclinical studies and some clinical intervention trials, selenium has emerged as a strong contender in the arena of cancer chemoprevention (6). In the Nutritional Prevention Trial, SY supplementation was associated with a reduction in PCa development (7, 8). The form of selenium has been shown in both clinical and preclinical studies to be an important determinant in chemopreventive efficacy. In the recently conducted Selenium and Vitamin E Cancer Prevention Trial (SELECT), selenomethionine (SM) was tested for its activity against PCa (9). Unfortunately, this trial was stopped prematurely because, in part, of the lack of a protective effect of SM against PCa and a non-significant increase in type II diabetes. SM, when used in animal models, demonstrated either little or no activity in the chemoprevention of PCa (6,10, 11). In addition to SM, selenium-enriched yeast contains other forms of selenium that appear to be more effective than SM. Clearly, there is an urgent need to develop more effective selenium-based agents and appropriate biomarkers that can be altered by selenium intervention in future clinical studies (12).
An important goal in the development of cancer prevention strategies is the identification of sensitive and selective markers, as well as characterization of the molecular mechanisms and pathways by which chemoprevention agents can interfere with the progression of normal cells to the first definable stage of cancer. Proteomic profiling can be used to identify proteins that are expressed differentially upon intervention by specific chemopreventive agents that are known to impact the disease process; such proteins have the potential to serve as chemoprevention markers and possibly even as markers of disease progression. Although, research in this area is in its infancy, several proteomic platforms have been used to identify differentially expressed proteins in normal and diseased prostate tissue specimens (13, 14). Furthermore, proteomic profiling has been used to identify changes in serum proteins associated with PCa (15-18). Clearly, this technology holds promise as a strategy for the identification of biomarkers that precisely reflect cancer progression. Such protein biomarkers could be used to monitor efficacy of therapeutic and chemopreventive agents without the need for expensive disease outcome measures. However, there have been few studies examining the effects of chemoprevention on proteomic profiles. Using human prostate cancer cell lines, we showed that synthetic and naturally occurring selenium compounds were capable but to a varied extent, to alter proteomic profiles (19). Protein profiling was used to monitor changes in the serum proteome of patients with clinically localized PCa receiving SM supplementation (20); supplementation revealed statistically significant proteomic pattern changes which indicate that this technology is sufficiently sensitive to monitor changes in protein profiles following dietary components (21-23). However, there have been no studies examining the impact of the form of selenium (SY) demonstrated to be effective in a previous clinical trial on proteomic profiling (7). The key objective of this study was to identify a panel of proteins that show differential expression in SY supplemented healthy AA and Caucasian men and determine by gene ontology classification if the differentially expressed proteins may play any role in the multi-step carcinogenesis process.
Materials and Methods

Study design and subjects. We had previously conducted a randomized, double-blinded, placebo-controlled clinical trial of SY supplementation (24) and stored serum samples were used for proteomic analysis in the current investigation. Briefly, a total of thirty six AA and Caucasian healthy adult males (19–43 years of age), non-smokers with comparable body mass index were enrolled. All subjects were randomized into either SY arm (247 μg Se/day, 17 men) or the placebo arm (19 men). Baseline data were collected on demographics, lifestyle habits, and usual dietary practices. Blood samples were collected at baseline and at 3, 9, and 12 months. Subjects from both arms were placed on placebo at 9 months, and final blood samples were collected at 12 months. At the 12 month time point, blood samples were collected to determine whether the effect of SY supplementation on parameters measured is reversible.

Albumin and major protein depletion from serum. Albumin along with five other abundant proteins including gamma globulins was depleted from all the serum samples with the ASK™ albumin removal Kit (ITSI Biosciences, Johnstown, PA) prior to analysis by two dimensional difference in gel electrophoresis (2D-DIGE). Briefly, each serum sample was thawed on ice, centrifuged at 10,000 xg for 5 min to clarify the serum. Then 100 μl of the supernatant was transferred to a clean microfuge tube and 400 μl of ice cold buffer-1 (0.1% potassium phosphate monobasic, 0.2% potassium phosphate dibasic) was added. The tube was vortexed, incubated at −20°C for 30 min and centrifuged at 4°C for 5 min at 15,000 xg. The supernatant was carefully removed and discarded. To wash the pellet, 1ml of chilled buffer-2 (0.2% potassium phosphate monobasic, 0.5% potassium phosphate dibasic) was added followed by 5 μl of ProPreCip™ (1% proteomics grade Ficoll; ITSI Biosciences, Johnstown, PA) a precipitation booster. After vortexing, the tube was incubated at -20°C for 15 minutes and centrifuged at 4°C for 5 min at 15,000 xg. The supernatant was discarded and the wash process repeated. After
the second wash, the supernatant was removed and the pellet resuspended in lysis buffer (7M urea, 2M thiourea, 4% Chaps, 0.5% NP-40, 5 mM magnesium acetate, 30 mM Tris-HCl, pH 8.5) using a disposable plastic pestle. The resuspended sample was incubated on ice for 30 min, with 4 vortexes and centrifuged at 15,000 x g for 10 min. The supernatant was transferred to a fresh tube and placed on ice until analyzed on the same day. Total protein content was determined with the ToPA™ protein assay kit and Bradford protocol (ITSI Biosciences, Johnstown, PA) using BSA as standard.

2D-DIGE analysis. For 2D-DIGE, 50 µg each of total protein isolated from yeast-supplemented and placebo samples were labeled with 200 pmoles of Cy3 or Cy5, respectively. Dye swapping was performed to compensate for any slight difference in signal intensity due to batch-to-batch variations in Cy dye intensity. An aliquot of equal concentration of proteins from all the samples (supplemented and placebo) were mixed and 50 µg labeled with Cy2 to obtain a universal internal control (25). The Cy2, Cy3 and Cy5 labeled samples were mixed to obtain a total of 150 µg of total protein and co-separated by isoelectric focusing (IEF) with pH 3-10 linear Immobiline Drystrips (GE Healthcare, Piscataway, NJ). IEF was for a total of 65,500 volt hours on an IPGphor (GE Healthcare, Piscataway, NJ). The focused strips were equilibrated for 15 min in SDS equilibration buffer containing 1% DTT, and then equilibrated in SDS equilibration buffer containing 2.5% iodoacetamide for 15 min (25). The strips were subsequently placed on 24 cm x 20 cm, 12.5% SDS-PAGE gels and electrophoresed at 15 watts per gel for about 4.5 hours.

Image acquisition and analysis. After 2nd dimension electrophoreses, all the gels were scanned on a DIGE-enabled Typhoon Trio Variable Mode Digital Imager (GE Healthcare, Piscataway, NJ) using the excitation/emission wavelengths for Cy2 (488 nm/520 nm), Cy3 (532 nm/580 nm) and Cy5 (633 nm/670 nm). The images obtained (3 per gel) were imported into the Biological Variation Analysis (BVA) module of DeCyder software (Version 6.0, GE Healthcare, Piscataway, NJ) for global normalization and identification of differentially abundant spots.
between supplemented and placebo samples with False Discovery Control set at 5%. All protein spots automatically detected in DeCyder after normalization that displayed mean statistically significant difference \((p \leq 0.05)\) in spot intensity between SY-treatment groups and placebo-control, and that were detected in at least 75% of gels were considered candidate differentially abundant protein spots. Each spot was manually inspected and verified before inclusion in our protein-of-interest (POI) list. A subset of the POI were selected for identification by tandem mass spectrometry.

**Candidate spot picking and processing.** To obtain enough protein/spot that will allow in-gel digestion and peptide sequencing by LC/MS/MS we ran a semi-preparative 2D-PAGE (“picking gel”) using a total of 300 \(\mu\)g of protein without Cy Dye labeling. After 1\(^{st}\) and 2\(^{nd}\) dimension electrophoresis using the parameters described above, the gel was stained in the dark for 1 hr with SyproRuby (Invitrogen, Carlsbad, CA) with shaking. The stained gel was rinsed with distilled water and scanned with the Typhoon Trio using the excitation (532 nm) and emission (610 nm) filters recommended for SyproRuby. The “picking gel” image was imported into the BVA module of DeCyder, matched to the 2D-DIGE image to identify the spots of interest and a pick-list was generated. The selected spots were picked with the Ettan Spot Picker (GE Healthcare, Piscataway, NJ) and in gel digested overnight with trypsin (Sigma, St. Louis, MO) at room temperature using the Ettan Spot Digester (GE Healthcare, Piscataway, NJ). The in-gel digested samples were extracted in 50 \(\mu\)l of 50% acetonitrile/0.1 % formic acid for 20 minutes (25), dried down completely at 45°C and stored at -20°C until sequenced by LC/MS/MS.

**Identification of proteins by LC/MS/MS.** All LC/MS/MS analyses were performed with the ThermoElectron ProteomeX Workstation (Thermo Corporation, San Jose, CA), which includes a Surveyor HPLC and an LCQ DecaXP Plus electrospray-ion-trap mass spectrometer. The dried down tryptic digested peptides were re-suspended in 15 \(\mu\)l of 1% formic acid and 10 \(\mu\)l of the mixture were loaded onto a Thermo Hypersil-Keystone BioBasic C18 column (0.18 x 100 mm)
and chromatographed with the Surveyor HPLC system. The peptides were separated using two buffer systems: Buffer A (0.1% formic acid in water) and Buffer B (0.1% formic acid in acetonitrile) at a flow rate of 4 μl/min. The gradient used was 2-50% Buffer A in 45 min. As peptides eluted from the column, they were subjected to ESI-lon-trap ms as follows: i) single full ms scan to determine the masses of analytes and ii) tandem ms of the three biggest peaks from the preceding full scan to obtain the sequence information. This process was repeated every 15 sec during the gradient. All the ms/ms spectra obtained were searched against the NCBI non-redundant protein sequence database using the SEQUEST computer algorithm to establish the protein identity (26).

**Gene ontology classification.** The 11 proteins of interest were assigned molecular functions, biological processes and cellular components according to the unified Gene Ontology (GO) Consortium classification (27), to determine their validated or putative role in several cancers including prostate cancer. The GO categories assigned to the proteins of interest were determined by using the gene ID of each protein to search the GO database to obtain the function, process and component assigned to each protein (28).

**Two-dimensional western blot analysis.** 130 μg of protein was added to an appropriate volume of rehydration buffer (8M urea, 2% CHAPS, 0.5% IPG buffer (pH 3–10), 0.28% DTT, and 0.002% bromophenol blue) for a final sample volume of 250 μl. Samples were loaded onto 13 cm IPG strips with a pH gradient of 3-10 (Immobiline DryStrip, GE Healthcare, Piscataway, NJ). Isoelectric focusing (IEF) was carried out in ceramic strip holders under paraffin oil using the IPGphor IEF system (GE Healthcare, Piscataway, NJ). After first dimension separation, strips were equilibrated in an SDS equilibration solution (50 mM Tris-HCl, pH 8.8, 6M urea, 30% glycerol, 2% SDS, 1% DTT, and 0.002% bromophenol blue), loaded on the top of a 10% polyacrylamide gel, and second dimension separation was carried out by SDS-PAGE. Sample proteins were then transferred to nitrocellulose membranes. Membranes were blocked using...
5% milk solution and probed with mouse monoclonal primary antibodies (1:1000) for human AAT or human clusterin from Abcam Inc., Cambridge, MA. Protein spots were detected by chemiluminescence using the ECL™ Western Blotting Analysis System (GE Healthcare, Piscataway, NJ).

**AAT ELISA.** The serum samples were diluted (10,000 fold) prior to determination of AAT levels using an enzyme-linked immunosorbent assay (GenWay Biotech, Inc., San Diego, CA) according to the manufacturer’s instructions. Each sample was measured in duplicate. A standard curve was constructed with each assay (range: 7.8 – 500 ng/ml) and AAT values for each serum sample was calculated in g/L.

**Statistical analysis.** Data are presented as Mean ± SE for AAT and PSA levels. The serum AAT levels among placebo and SY-supplemented individuals were statistically evaluated as a function of race and time using students ‘t’ test and the p values <0.05 were considered statistically significant.

**Results**

**Global proteomic profile of serum from SY supplemented men.** Several protein spots were detected by 2D-DIGE using a 3-10 pH range of ampholytes in the serum samples from SY-supplemented healthy men. A total of 1496 candidate protein spots were detected in the BVA module of DeCyder. Following filtering using the p-value ≤0.05 as the cut off, we identified 8 spots that were up-regulated and 3 spots that were down regulated. The 11 candidate spots were picked and identified by LC/MS/MS (Table 1). In general, the selected proteins are redox-sensitive or involved in regulation of redox status. Gene Ontology (GO) classification revealed other functions and processes and indicated that 7 out of 11 of the proteins have been reported to be involved in the multi-step carcinogenesis process (Table 2). AAT (Figure 1A, B) and CLU (Figure 1C) are of particular interest because literature data clearly demonstrates their
involvement in the carcinogenesis process. We therefore independently confirmed their expression profile by 2D-western blot analysis. Figure 1A shows the possible isoforms (indicated by arrows) of AAT being reduced in sera from SY supplemented individuals after 9-months of treatment; however, CLU was up regulated after SY-supplementation.

AAT was further examined in three representative serum samples from placebo and SY supplemented healthy men. Our rationale for further analysis of AAT was based on literature data demonstrating that levels of AAT vary with race and are correlated with PSA levels (29).

**Influence of SY supplementation on AAT levels.** Serum samples from placebo and SY-supplemented healthy men were examined for AAT levels at baseline, and at 3 and 9 months after supplementation in addition to analyzing samples at 12 months from the start of the study. The AAT ELISA showed that there was a slight (nonsignificant) elevation at 3 months for the placebo group but thereafter the AAT levels appear to decline at 9 and 12 months (Figure 2). The SY supplemented group, however, showed a significant decrease in the AAT levels at 9 months (p<0.05) when compared to the baseline levels (Figure 2). However, at 9 months the difference observed in mean AAT between placebo and supplemented groups was not significant. After a 3 month washout period (12 month time-point) AAT levels in the selenium supplemented group were comparable to baseline. Overall, AAT levels in supplemented individuals were inversely correlated with changes in selenium levels from baseline at 3, 9 and 12 months with r values ranging from -0.47 to -0.59 (p<0.05).

Further evaluation of AAT levels indicated differences between Caucasians and AA. AAT levels for AA were consistently higher than Caucasians at any given time point during the study. Following supplementation, AAT levels in Caucasians were significantly lower (p<0.05) than those compared to AA at 3 months (Figure 3A). Moreover, in AA the AAT levels were lowered significantly by 3 and 9 months of SY supplementation compared to baseline (p<0.05), while in Caucasians the levels of AAT were significantly (p<0.05) reduced only after 9 months.
where they remained lower even after 12 months (p<0.05) (Figure 3A). Interestingly, since SY-supplementation was terminated after 9 months, the levels of AAT in AA started to recover at 12 month interval while in Caucasians these levels remained significantly lower (p<0.05) than those at baseline. SY appears to equally reduce PSA levels in both Caucasians and AA (Figure 3B).

**Discussion**

In the present study, proteomic analysis of serum from participants of our previous trial (24) revealed 11 proteins that were significantly altered after 9-months of SY supplementation, all of which are redox sensitive or involved in the regulation of redox status. The GO classification of the selected proteins revealed that 7 out of the 11 play a role in cancer development including prostate cancer (Table 2). For example, Alpha-1B glycoprotein is over expressed in pancreatic cancer (30), haptoglobin is differentially expressed in adenocarcinoma of the uterus (31) and transthyretin expression is reduced in lung cancer (32) and in cholangiocarcinoma (33). Additionally, Transferrin and Angiotensin precursor are implicated in lung cancer (34) and renal cell carcinoma (35), respectively. Our hypothesis that selenium may inhibit oxidative stress and other markers of risk for PCa due to covalent interactions of selenium with thiol containing redox-sensitive proteins is supported in part by our previous study (36). However, future studies are required to determine the basis for SY alteration of the redox-sensitive proteins identified in this study.

Of particular interest was our finding that AAT and CLU were among the proteins most affected by SY, as these have been previously linked to the development of PCa (37-39). CLU, a glycoprotein, is believed to be involved in many diseases including cancer (reviewed in 37). CLU gene is a regulator of apoptosis, cell-cell interactions, protein stability, cell signaling, proliferation, and transformation. In humans, the CLU gene is located on chromosome 8 in a region that is frequently deleted in prostate cancer (8p21-p12)(37). Numerous studies have
suggested that CLU expression is altered during different phases of prostate tumorigenesis with both increases and decreases being observed depending on the model systems being examined (38). However, CLU appears to be consistently down-regulated during early stages of prostate cancer progression (39). While the mechanisms by which CLU may impact carcinogenesis is not clear, studies have indicated that CLU expression is subject to regulation via epigenetic mechanisms during prostate cancer development (39). Our results clearly showed that SY enhanced a form of CLU protein expression in healthy men. While there is little data available regarding the impact of chemopreventive agents on CLU expression, previous studies with patients bearing high grade intraepithelial neoplasia (HGPIN) and supplemented with green tea catechins (GTCs) support a putative role for CLU in the prevention of HGPIN (40, 41). Collectively, our results on the impact of SY on CLU levels in healthy subjects and those reported on the effect of GTCs in high risk HGPIN population are encouraging and provide clinical opportunities to monitor CLU as a biomarker in future clinical intervention trials.

Our findings that AAT levels are significantly decreased by SY and are highly correlated with an increase in blood selenium levels are consistent with a possible reduction in PCa risk. Elevations in serum levels of AAT, a marker of inflammation, in PCa patients are well documented. Serum levels of AAT are increased by inflammation as well as infection (42, 43). Blood levels of AAT are tightly regulated and increased in PCa patients. In fact, levels of various proteins including AAT correlated with disease severity and decreased in patients with favorable response to treatment (44, 45). Similarly, a decrease in PSA levels is usually observed in PCa patients who respond to treatment, whereas increased levels are indicative of poor prognosis (46, 47). It is thought that inflammation of the prostate or prostatitis may lead to PCa by mechanisms that remain to be fully elucidated. Furthermore, it was reported that men with PCa associated with metastasis had higher serum levels of AAT than those with less advanced disease (48).
In the current study, we showed that AAT levels for AA were higher than that for Caucasians at any given time point during the trial. This result is consistent with the literature data demonstrating that AA have higher levels of AAT as well as PSA than Caucasians (29). In addition, an age associated increase in AAT levels, especially in AA, was also reported (29).

Re-analysis of our previous pilot selenium trial data (24) indicated that the increase in plasma selenium by SY supplementation was much lower in AA than in Caucasians (unpublished data*). These results are consistent with recent analysis of US population data from National Health and Nutrition Examination Survey (NHANESIII) (49) where selenium levels were about 6% lower in AA than in caucasians after adjustment for known predictors of serum selenium. In the present study we showed that SY supplementation resulted in significantly lower AAT levels in caucasians than those compared to AA at a 3-month time point. Our results also demonstrate that the levels of AAT in AA recovered at 12 month faster than those found in caucasians. Collectively, these results suggest that lower selenium levels in AA (49, unpublished data*) may, in part, contribute to their higher rate of cancer, particularly, PCa underscoring the need to tailor future clinical chemoprevention trials according to race.

Independent of the mechanism that can account for the alterations of levels of proteins identified in this study including AAT and CLU by SY, we propose that in addition to the standard PSA test, both proteins are potential candidate biomarkers that can be employed in pilot clinical trials using selenium.

At present, our knowledge on the mechanisms that may account for cancer prevention by various forms of selenium is based primarily on animal model studies and assays in cultured prostate cancer cells (6, 19, 50). Unfortunately, how such knowledge can be applied to humans is unclear and this void presents a great challenge in the design of future clinical trials. At present a clinical study is currently being conducted in our laboratory aimed at comparing the effect of SM and SY under identical conditions, on protein profiles and on biomarkers of PCa.
risk in an ethnically mixed cohort of men. Clearly these types of clinical pilot studies with chemopreventive agents such as selenium need to be pursued prior to entering into long-term, expensive phase III clinical chemoprevention trials.


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Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

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**Figure legends**

**Figure 1.** 2D-western blots for serum AAT and CLU. The 9 month (t = 9 Mo) time point was selected to confirm the presence of AAT and CLU proteins. (A) AAT isoforms (arrows) in sera of healthy men supplemented with plain yeast (placebo) or selenium-enriched yeast (SY) for 9 months. Western blots of three representative samples. (B) AAT level comparison between baseline and 9-months treatment, note AAT was decreased several fold in SY supplemented
sample with no change in AAT for placebo sample. (C) Representative western blot for confirmation of CLU, note the higher expression in SY sample.

**Figure 2.** Change in AAT from baseline levels in sera of healthy men supplemented with plain yeast (placebo) and SY. The AAT levels in healthy men were significantly reduced following 9 months of SY-supplementation as compared to baseline and 3 months (p<0.05).

**Figure 3A.** AAT levels in AA and Caucasian men following SY supplementation. The AAT levels were significantly reduced (p<0.05) in SY-supplemented AA men at 3, 9 and 12 months compared to baseline. Levels of AAT in sera of caucasians were significantly reduced as compared to AA men at 3 months of SY supplementation.

**Figure 3B.** PSA levels of SY-supplemented AA and Caucasian men. PSA levels were equally reduced in AA and Caucasian men during SY supplementation.
Table 1. Characteristics of serum proteins identified from SY supplemented and placebo individuals following 2D-DIGE and LC/MS/MS analysis.

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<th>Spot No.</th>
<th>Identified Protein</th>
<th>Accession No.</th>
<th>Supplemented/Placebo (Fold Change)</th>
<th>p value</th>
<th>Mass (Daltons)</th>
<th>pI</th>
<th>Peptide hits (unique)</th>
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<td>412</td>
<td>Alpha 1B-glycoprotein</td>
<td>21071030</td>
<td>+1.29</td>
<td>0.037</td>
<td>54,220</td>
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<td>425</td>
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<td>0.046</td>
<td>77,001</td>
<td>6.00</td>
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<tr>
<td>538</td>
<td>Complement component 4B proprotein</td>
<td>4502501</td>
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<td>192,678</td>
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<td>39,567</td>
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<tr>
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<td>57,797</td>
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a The proteomic analysis was performed on serum of subjects supplemented with SY or placebo for 9 months.
b National Center for Biotechnology Information (NCBI)
c Fold change (for e.g., +2.00 implies a doubling of the protein in the SY vs placebo group, and a -1.66 means 66% lower protein in SY vs placebo group).
d Isoelectric point
e Number of peptides that uniquely match the protein identified by mass spec analysis
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<th>Name</th>
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<th>Component</th>
<th>Role in or altered in cancer+</th>
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<td>No data is available</td>
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<td>Ferric iron binding, metal ion binding, protein binding</td>
<td>Cellular iron, ion transport, iron ion transport</td>
<td>Apical plasma membrane, Extracellular region</td>
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<td>Complement activation, classical pathway inflammatory response innate immune response</td>
<td>Extracellular region, extracellular space</td>
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<td>NAD or NADH binding, Isocitrate dehydrogenase (NAD+) activity, Magnesium ion binding oxidoreductase activity</td>
<td>2-oxoglutarate metabolic process. NADH metabolic process. Carbohydrate metabolic process</td>
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<td>Cluster isoform 1 (CLU)</td>
<td>Misfolded protein binding, protein binding</td>
<td>Anti-apoptosis cell death complement activation, classical pathway</td>
<td>Aggresome, extracellular region, extracellular space</td>
<td>YES</td>
</tr>
<tr>
<td>Keratin 1</td>
<td>Protein binding, receptor activity, structural constituent of cytoskeleton, sugar binding</td>
<td>complement activation, Lectin pathway, epidermis development, fibrinolysis, regulation of angiogenesis, response to oxidative stress</td>
<td>Cytoskeleton, intermediate filament, keratin filament, membrane, plasma membrane</td>
<td>NO</td>
</tr>
<tr>
<td>Transthyretin</td>
<td>Hormone activity, protein binding</td>
<td>Transport</td>
<td>cytoplasm, extracellular region</td>
<td>YES</td>
</tr>
<tr>
<td>Alpha-1 antitrypsin (AAT)</td>
<td>Peptidase inhibitor activity, protein binding</td>
<td>Acute-phase response, response to estradiol stimulus, response to hypoxia.</td>
<td>Extracellular region, extracellular space, extracellular matrix</td>
<td>YES</td>
</tr>
<tr>
<td>Angiotensin precursor</td>
<td>Acetyltransferase activator activity, growth factor activity</td>
<td>Negative regulation of cell growth, negative regulation of cell proliferation</td>
<td>Cytoplasmic part, extracellular region, extracellular space, soluble fraction</td>
<td>YES</td>
</tr>
<tr>
<td>Albumin precursor</td>
<td>DNA binding, Antioxidant activity, Chaperone binding, Copper ion binding</td>
<td>Negative regulation of apoptosis.</td>
<td>Extracellular region, extracellular space</td>
<td>MAYBE</td>
</tr>
</tbody>
</table>

*Information presented under function, is only a small number of the total entries found.
+Obtained from published papers. See text for more details.
Figure 1. Sinha et al
Figure 2. Sinha et al
Figure 3A. Sinha et al
Figure 3B. Sinha et al
Selenium responsive proteins in sera of selenium-enriched yeast supplemented healthy African American and Caucasian men

Raghu Sinha, Indu Sinha, Nicole Facompre, et al.

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