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The Urine Proteome as a Biomarker of Radiation Injury:

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Abstract

Terrorist attacks or nuclear accidents could expose large numbers of people to ionizing radiation, and early biomarkers of radiation injury would be critical for triage, treatment and follow-up of such individuals. However, no such biomarkers have yet been proven to exist. We tested the potential of high throughput proteomics to identify protein biomarkers of radiation injury after total body X-ray irradiation in a rat model. Subtle functional changes in the kidney are suggested by an increased glomerular permeability for macromolecules measured within 24 hours after TBI. Ultrastructural changes in glomerular podocytes include partial loss of the interdigitating organization of foot processes. Analysis of urine by LC-MS/MS and 2D-GE showed significant changes in the urine proteome within 24 hours after TBI. Tissue kallikrein 1-related peptidase, cysteine proteinase inhibitor cystatin C and oxidized histidine were found to be increased while a number of proteinase inhibitors including kallikrein-binding protein and albumin were found to be decreased post-irradiation. Thus, TBI causes immediately detectable changes in renal structure and function and in the urinary protein profile. This suggests that both systemic and renal changes are induced by radiation and it may be possible to identify a set of biomarkers unique to radiation injury.

Keywords

Radiation injury; Radiological terrorism; Nuclear accidents; Biomarkers; Urine proteome

1 Introduction

Ionizing radiation causes injury to normal tissues in a tissue- and dose-specific manner. Some tissues, including the gastrointestinal tract, lungs and kidneys are more sensitive to radiation.

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Radiation nephropathy is a well-known complication of the total body irradiation (TBI) that is often used prior to bone marrow transplantation (BMT) [1], and is also seen after therapeutic applications of antibody-bound radioligands [2]. Although early signs of renal injury have not been defined, subsequent renal dysfunction is indicated by proteinuria, high blood pressure and increased BUN that occur months to years after TBI [3].

The threat of a radiological detonation by terrorists or an accident at nuclear facilities has become a subject of general concern [4,5]. Acute injuries that immediately follow a radiation event and lead to rapid loss of health and life have been the primary focus of these concerns. However, there is increasing recognition of the potential damage and late manifestations caused by relatively low doses of ionizing radiation [4-6]. A much larger number of people are expected to be exposed to low doses than to instantly lethal doses of radiation following a nuclear explosion [4,7,8]. Such catastrophic circumstances will demand that medical personnel be able to make critical triage decisions based on limited information. Rapid detection of radiation injury by high throughput automated equipment or a point of care assay will be needed for early intervention to prevent loss of organ function and mortality [9]. The required basic information for a better understanding of initial injury, mechanisms leading to late manifestation of disease and preparedness for early intervention can be obtained only through the study of animal models of radiation exposure.

We have developed a rat model of BMT nephropathy that develops proteinuria at about 6 weeks post-TBI as the first overt sign of renal injury [10,11]. We have also reported increased glomerular albumin permeability at one hour after TBI of rats [12]. We proposed that altered glomerular permeability to macromolecules indicates early renal injury, which is undetectable by conventional methods (e.g. routine urinalysis), and additionally that irradiation causes immediate changes in renal tubular function. Thus, subtle renal injury caused by TBI may alter the urinary protein profile without causing proteinuria detectable in standard testing. These qualitative changes in urinary protein composition require sensitive techniques for detection and identification. Thus, urine may provide a valuable source of biomarkers for radiation injury. The fact that urine can be obtained non-invasively makes it an especially attractive candidate biofluid for monitoring radiation injury during emergencies.

Proteomic analysis using liquid chromatography-mass spectrometry (LC-MS/MS) and bioinformatics is a very sensitive high throughput approach for protein identification in complex biological materials. Qualitative and quantitative changes in the urinary proteins in large numbers of samples can be determined by high throughput techniques. LC-MS/MS based proteomics and related approaches have been increasingly employed to study renal and other diseases [13]. We have applied these techniques, as well as studies of glomerular function and ultrastructure, to analyze the radiation induced changes observed at 24 hours after TBI.

2 Materials and methods

2.1 Animals

The rats used in the study were eight weeks-old male WAG/Rij/MCW that were bred and housed in a moderate security barrier at the Medical College of Wisconsin, Milwaukee. Animals were maintained in the Biomedical Resource Center of the Medical College of Wisconsin, which is fully accredited by the American Association of Accreditation of Laboratory Animal Care. The protocols used in the study had been reviewed and approved by the Institutional Animal Care and Use Committee and the Biohazard Committee of the Medical College of Wisconsin.

2.2 Irradiation

Conscious animals were given total body irradiation (TBI) with orthovoltage X-rays. Ten Gy X-irradiation was given in one fraction at a dose rate of 1.95 Gy/minute. Control rats were sham irradiated.

2.3 Urine collection and measurement of urinary protein and creatinine in rats

Rats were placed individually in metabolic cages for 24 hours and urine was collected in clean plastic tubes. Fresh urine samples were centrifuged (3000xg) and the supernatant was used to determine total protein using a reagent kit based on Lowry's method (BioRad, Hercules, CA) and urine creatinine using a method based on Jaffe reaction (Teco Diagnostics, Anaheim, CA) [14].

2.4 Kidney dissection and isolation of glomeruli

Kidneys were removed via an abdominal incision after the animals were anesthetized using isoflurane vapor (Phoenix Pharmaceuticals Inc., St. Joseph, MO). Glomeruli were isolated using an established sieving technique. After the kidney capsule was removed, fine fragments of the superficial 1 to 2 mm renal cortex were prepared and passed through consecutive screens of 80, 140, and 200 mesh. Glomeruli were recovered from atop the 200 mesh screen. Isolation of glomeruli was carried out at room temperature in a medium which contained, in mmol/L, sodium chloride, 115; potassium chloride, 5; sodium acetate, 10; dibasic sodium phosphate, 1.2; sodium bicarbonate, 25; magnesium sulfate, 1.2; calcium chloride 1; glucose, 5.5; L-alanine, 6; sodium citrate, 1; and sodium lactate, 4. Bovine serum albumin (BSA), 5 g/dL, was included in the medium (isolation/incubation buffer) as an oncotic agent. The pH of the medium was adjusted to 7.4. The oncotic pressure was measured using a membrane colloid osmometer (Model 4100; Wescor Inc., Logan, UT).

2.5 In vitro glomerular albumin permeability assay

Glomeruli from control and experimental rats were incubated at 37°C for the duration of each experiment in isolation medium containing 5% BSA. After incubation, glomeruli were held on a glass surface and initial images of at least 5 glomeruli were recorded by videomicroscopy. The medium was changed from 5% to 1% BSA to generate an oncotic gradient across the glomerular capillary wall causing movement of fluid into the capillaries and an increase in glomerular size. The final image of each glomerulus was recorded after 60 seconds. As shown in Figure 1A, the diameter of each video image was measured along four diameters 45° apart and the average diameter was used to calculate initial and final volumes (V_{initial} and V_{final}). The change in volume due to oncotic gradient of each glomerulus (ΔV) was calculated as: $\Delta V = [(V_{\text{final}} - V_{\text{initial}}) / V_{\text{initial}}] \times 100\%$.

The effective force produced by an albumin oncotic gradient across the capillary wall (about 16 mm Hg under experimental conditions used) is calculated using the change in glomerular volume. The reflection coefficient (σ) of a membrane measured in relation to a given solute is defined by the ratio of the oncotic force exerted by that solute to its theoretical oncotic force. σ is a function both of the membrane and of the physical characteristics of the solute including molecular radius, configuration and charge. Albumin reflection coefficient (σ_{alb}) was calculated as: $\sigma_{\text{alb}} = \Delta V_{\text{experimental}} / \Delta V_{\text{control}}$. Convective permeability (P_{alb}) was calculated as: $P_{\text{alb}} = 1 - \sigma_{\text{alb}}$. When σ_{alb} is zero, albumin exerts no oncotic pressure and P_{alb} equals 1.0. When σ_{alb} is 1.0, albumin exerts a force equal to that calculated from the chemical gradient across the membrane and P_{alb} equals zero [12,15,16].

2.6 Scanning electron microscopy (SEM)

Isolated glomeruli were fixed using modified 2% glutaraldehyde in 0.1 M phosphate buffer, rinsed several times with PBS and post fixed with 1% osmium tetroxide in 0.1M phosphate buffer for 1 hour. After rinsing with PBS for 15 minutes, glomeruli were dehydrated using 70% ethyl alcohol for 15 min, 95% ethyl alcohol for 15 min and 3 changes of 100% ethyl alcohol for 10 min Each. They were then dried, mounted on aluminum stubs with adhesive tabs and sputter coated. Glomeruli were viewed using AMRAY 1000A (Bedford, MA) scanning electron microscope at the Electron Microscopy Center, Department of Anatomy and Cell Biology, Indiana University School of Medicine, Indianapolis, IN.

2.7 Two-Dimensional Electrophoresis

Urine samples collected as described above were immediately centrifuged (3000×g) to remove debris. Equal amounts of total protein from each sample were pooled and aliquots containing 200 µg protein were mixed with a 2 ml rehydration buffer (8M urea, 2% CHAPS zwitterionic detergent, trace of bromophenol blue), transferred to Centricon-3 centrifugal filter unit (Millipore, Billerica, MA) and centrifuge at 5000×g for 3 hours at 4°C. The residual sample was made up to 200 µl with rehydration buffer, inverted and transferred to a collection tube by centrifugation at 5000×g (4°C, 10 minutes).

Immediately before the first dimensional electrophoresis, samples were mixed with freshly prepared dithiothreitol (DTT, 7mg/2.5mL) and 0.5% IPG buffer, pipetted into lanes and covered with 11 cm long immobilized pH gradient (IPG) strips (pH3-10, gel-side down). After rehydration IPG strips were covered with mineral oil and electrofocusing was carried out for 8000 volt hours. IPG strips were inverted (gel-side up) placed in an equilibrating buffer for 10 minutes (50mM Tris-HCl, pH 8.8, 6M urea, 30% glycerol, 2% SDS, trace bromophenol blue and freshly added 10 mg DTT in 1ml buffer. Next, equilibrated IPG strips were placed over pre-cast Criterion SDS-polyacrylamide gel (Criterion, BioRad) and electrophoresed for 1.5 hours at 150V for size-based second dimension electrophoresis of proteins. Proteins were visualized using a silver-staining protocol.

2.8 Fractionation and mass spectrometry of urinary proteins

Pooled urine samples (200 µg protein from each rat) were concentrated and desalted using a Centricon-3 centrifugal filter unit (Millipore, Billerica, MA). Samples were mixed with a solution of 8M urea, 2% CHAPS, 20 mM DTT, and 2% pH 3-10 Biolyte ampholyte (BioRad, Hercules, CA) to a final volume of 2.7 ml and fractionated into 10 samples (pH range 3 - 10) by isoelectric focusing using a MicroRotor (BioRad, Hercules, CA). Each fraction was exchanged into 100 mM ammonium bicarbonate buffer, digested with trypsin (Promega, Madison, WI), dried and resuspended in 6M guanidine-HCl, 5mM potassium phosphate, pH 6.5, purified and concentrated using a C₁₈ ZipTip (Millipore, Billerica, MA) before analysis by LC-MS/MS. Samples were analyzed by nanospray-LC-MS/MS using an LTQ linear ion trap mass spectrometer (ThermoFisher, Waltham, MA).

2.9 Computational analysis

The resulting MS/MS spectra were searched against the rodent portion of the UniProt database using a two-stage process. MS/MS spectra peak lists (.*dta* files) were extracted from the instrument produced .RAW data files using the *extract_ms* program (ThermoFisher, Waltham, MA) with the following key parameters: B 600, T 3500, M 1.4, S1 G1, E 250. The MS/MS data was searched against the rodent subset of the UniProt database (release 54.0) with a version of the *Sequest* (V.12) program adapted for use on the JS-20 processor (IBM, Armonk, NY) in a cluster environment (ThermoFisher, Waltham, MA) using the following key parameters. For the first pass search: Enzyme Trypsin [KR], peptide tolerance 2.5 AMU, fragment ions

tolerance 1.0 AMU, differential modifications: M 16, C 57. For the second pass search: Enzyme No enzyme, peptide tolerance 2.5 AMU, fragment ions tolerance 1.0 AMU, differential modifications: MHP 16, C 57, K 1, R -47. The resulting search results (*.out files*) were parsed and the peptide and protein probabilities calculated using the *Epitomize* program (Medical College of Wisconsin, Milwaukee, WI) and the protein hits with probability scores > 0.6 from each search were saved. The protein hits from the individual sub-fractions derived from the original pooled urine samples were combined using the *Visualize* program (Medical College of Wisconsin) and revised protein probability scores calculated based on the observation of proteins in multiple runs, and proteins with a probability score of 0.90 retained. By searching similar data with decoy databases, it was found that this probability score corresponds to a False Discovery Rate (FDR) of 7.5%. These composite results from different time points were then quantitatively compared using the *Visualize* program and the ratios of protein abundance were exported for further analysis by *Excel* (Microsoft, Redmond, WA).

2.10 Statistical analysis

P_{alb} , total protein and creatinine values are expressed as mean $\pm$ SEM. Values of groups were compared using t test. Significance of difference between groups was expressed as *P* value.

3 Results

3.1 Glomerular albumin permeability (P_{alb}) increases after total body irradiation (TBI)

P_{alb} was determined at 15, 30, 60 minutes or 24 minutes after TBI to assess early subtle changes in renal function. Figure 1B shows that P_{alb} at 15 minutes, 60 minutes and at 24 hours were significantly higher than the respective controls ($P < 0.001$, $n = 15$ glomeruli, 3 rats in each group).

3.2 Scanning electron microscopy of glomeruli

Radiation-induced changes in glomerular ultrastructure at 24 hours post-irradiation were studied by SEM. Figure 2 shows the urinary aspect of decapsulated rat glomerulus. Some areas of the glomerular capillary loops have lost the characteristic compact interdigitation between foot processes (highlighted by circle) indicating altered junctional attachments between adjacent foot processes required to constitute the slit junctions. Glomerular capillary diameter was reduced in some areas. Some podocyte cell bodies and primary processes appeared collapsed (indicated by arrowhead).

3.3 Urinary protein and creatinine (Up and Uc)

Urinary total protein (mg/mL $\pm$ SEM) in the irradiated group (11.8 ± 0.65 , $n = 5$) was statistically not different from the control group (13.7 ± 1.16 , $n = 5$). Urinary creatinine (mg/mL $\pm$ SEM) in the irradiated group (0.83 ± 0.04 , $n = 5$) was not different from that in the control group (0.9 ± 0.04 , $n = 5$).

3.4 Two-Dimensional Electrophoresis

Results of two-dimensional gel electrophoresis (2DE) followed by silver staining of urinary proteins are shown in Figure 3. TBI-induced increase in the number of urinary proteins is clearly visible.

3.5 LC-MS/MS and computational analysis

Urinary proteins were analyzed by LC-MS/MS after fractionation of each sample by isoelectric focusing to improve coverage and to decrease interference by abundant proteins. The MS/MS spectra were searched using a two-stage process. First, we limited the search to only fully tryptic peptides excluding all modifications. From this search, we produced a list of the proteins

that had a 0.60 or higher protein probability score and created a new database. Next, this small database was searched with unconstrained (non-tryptic) protease specificity and multiple post-translational amino acid modifications expected to be induced by radiation. This approach greatly reduces the time required to undertake the more detailed search and also raises the protein probability scores and lowers the false discovery rates [Halligan et al., manuscript in preparation]. Proteins were quantitated using the 'label-free' approach based on spectral counting i.e. the relative abundance of a protein is assumed to be proportional to the number of individual spectra that were determined to originate from all of the peptides identified from that protein [17,18].

3.5.1 Total number of proteins in the urine—Two-dimensional gel electrophoresis of urine samples suggested an increase in the number of proteins at 24 hours after irradiation (Figure 3). The proteins identified from the urine of the one-day post irradiation and sham irradiated control rats were compared using the spectral counting approach in which the number of spectra above the 0.6 peptide probability threshold assigned to each protein is compared between samples. Table 1 shows the list of the 299 proteins identified from the urine samples from both the irradiated and unirradiated rats with a protein probability score of at least 0.90, corresponding to a false discovery rate of 7.5% (data not shown). Table 1 also includes the ratios of scans assigned to each of the proteins identified in control and irradiated samples. In the urine from the irradiated animals, 188 proteins were of 2 fold or greater abundance compared to control animals and 76 proteins were found to be 2 fold decreased in the irradiated animals compared to control animals. The remaining 35 proteins were present approximately equally in both samples and considered to be unchanged.

3.5.2 Gene Ontology (GO) analysis—To better understand the changes in the global patterns of urine protein abundance caused by irradiation, proteins were divided into three groups: increased 2 fold or greater in irradiated urine, decreased 2 fold or greater in irradiated urine, or unchanged by irradiation. Each of the proteins in each of the samples was mapped to the GO (Gene Ontology) terms associated with it for each of the three GO categories: cellular localization/component, molecular function, and biological process [19].

Figure 4A shows the number of proteins (count) associated with each of the matching GO terms for cellular compartment localization. 25 GO cellular compartments were used in this analysis. Proteins from 5 compartments namely, membrane, extracellular region, nucleus, cytoplasm and cytoskeleton compartments, accounted for most of the urine proteins identified in both control and experimental urines. The ratio of increased/decreased proteins was more than 4-fold for the intermediate filament, apical plasma membrane, keratin filament, mitochondria, lysosomes, matrix and soluble fraction proteins. We interpret this to indicate an increased number of proteins excreted in the urine.

Figure 4B shows the number of proteins associated with each of the matching GO terms for molecular function. Protein binding molecules accounted for the highest protein count. The increased/decreased ratio protein counts was more than 4-fold for glycosylphosphatidylinositol (GPI) anchor binding, calmodulin binding, sugar binding, serine-type endopeptidase activity, protein dimerization, kinases activity, motor activity, structural constituents of the cytoskeleton, peptidase activity, nucleotide binding, ATP binding and calcium binding classes of proteins. One exception to the general increase in binding proteins was the group of DNA binding proteins where the increased/decreased protein count ratio was less than 1.

Figure 4C shows a graph of the number of proteins associated with each of the matching GO terms for biological process. Urinary levels of proteins from all GO classes of biological processes were affected by irradiation. Proteins in several biological processes, namely anti-apoptotic, muscle contraction, epidermis development, lipid metabolism, proteolysis,

oxidative stress, inflammation, cell proliferation, cell differentiation, cytoskeletal organization and intracellular signaling showed more than 4-fold change in the ratio of increased/decreased protein counts.

3.5.3 Urinary proteinases and proteinase inhibitors—GO analysis indicated that irradiation results in increased levels of proteinases in the urinary. Proteinases and corresponding proteinase inhibitors are important in cellular response to injury and several classes of these molecules are present in the circulation and renal cells. We decided to determine whether certain proteinases are specifically affected in irradiated rats. Figure 5A shows that urinary levels of a number of proteinases are increased at 24 hours after TBI. These proteinases represent both plasma and cellular origins. Most noticeable increase occurred in the levels of Kallikrein 1-related peptidase b24 precursor (Glandular kallikrein K24, Tissue kallikrein 24, gi|48428341). We also found that products of chymotrypsin-like activity are detectable in the 24 hour urine after TBI (data not shown). Figure 5B shows that the observed increase in urinary proteinases was accompanied by a decrease in several proteinase inhibitors including kallikrein binding serine protease inhibitor A3K precursor (SERPIN A3K, gi|266407). The observed increase in proteinases and a parallel decrease in proteinase inhibitors reflect the early molecular events initiated by TBI.

3.5.4 Markers of acute kidney injury—LC-MS/MS results were analyzed to determine the presence of markers of acute kidney injury namely, Kidney Injury Molecule-1 (KIM), N-acetyl-Beta-D-glucosaminidase, Neutrophil gelatinase-associated lipocalin (NGAL), Cystatin C and Interleukin-18 (IL-18) [20,21]. TBI caused a 3-fold increase in the levels of Cystatin C (108 scans) compared to the control (35 scans). However, KIM, neutrophil gelatinase, N-acetyl beta-D-glucosaminidase or IL-18 were not detected..

3.5.5 Changes in protein oxidation—Database searches of MS/MS spectra were performed for amino acid oxidation as a dynamic modification through reaction with the reactive oxygen species generated by radiation. Such modifications include the oxidation of methionine (M), histidine (H), lysine (K) and proline (P) as well as the formation of glutamic acid semialdehyde from arginine (R). As part of the database search, dynamic modifications of M +16, P + 16, H +16, K -1 and R -43, were also considered. The number of spectra of modified peptides that contributed to the set of confidently identified proteins (probability score > 0.9) containing each modification was determined and compared to the total number of spectra from each group of experiments (Table 2). As shown, modified amino acids were detected in both the irradiated and control urine samples. While most of the modifications were detected at similar frequencies, there was a decrease in oxidized methionine and a nearly 3-fold increase in oxidized histidine after TBI.

3.5.6 Plasma proteins in the urine after irradiation—We did not detect an increase in total urinary protein although 2D-GE showed an increase in the number of proteins after irradiation. These results suggested a decreased excretion of certain proteins. To test this hypothesis, we examined the levels of the most abundant plasma proteins in the urine of irradiated and control animals. Figure 6 shows a bar graph of the number of spectra assigned to the most abundant plasma proteins. Albumin, the most abundant plasma protein that accounts for approximately half of plasma proteins by mass, is clearly detected in both the irradiated and control urine samples. The absolute number of spectra assigned to albumin in irradiated pool is less than in the control pool and albumin dropped from the 8th most observed protein in control urine to the 14th most observed protein in irradiated urine. Similarly, the immunoglobulins, the next most abundant class of plasma proteins are also detectable in both irradiated and control urine samples and the absolute number of spectra and relative abundance of these proteins also appeared to decreased in the irradiated pool. In contrast to this finding,

urinary levels of the acute phase response protein, alpha-1-acid glycoprotein 1 (orosomucoid 1) were increased greater than 5 fold following TBI.

4 Discussion

The exposure of large numbers of people to radiation in a terrorist event would put extreme demands on resources and trained manpower. The development of early biomarkers of radiation injury would be critical for triage, treatment and follow-up of irradiated individuals after such an event. No such biomarkers have yet been proven to exist, but the urine proteome changes detected in this study may provide such markers. While it is critical to develop effective strategies for immediate intervention through better understanding of the initial injury and its progression, it is not possible to study the effect of ionizing radiation on normal human subjects. Thus, experimental animal studies must be the starting point for understanding the initial effects of ionizing radiation, progression of injury, and for evaluating the efficacy of intervention strategies. We have initiated these investigations using a rat model of radiation nephropathy. In order to use the urine as a source of biomarkers of radiation injury we proceeded to first demonstrate that non lethal doses (10 Gy) cause subtle but immediate changes in renal function and structure.

We have extensively used an *in vitro* assay of glomerular albumin permeability (P_{alb}) as a surrogate marker of early renal injury. Using this assay we have shown that cytokines such as TGF- β and TNF α , free radicals such as superoxide, antibodies to glomerular proteins or human plasma factor increase P_{alb} . Additionally, rat models of type 2 diabetes, puromycin aminonucleoside nephrosis and radiation nephropathy show increased glomerular permeability prior to the onset of albuminuria [22,23]. Thus, increased P_{alb} is a sensitive indicator of the initial changes in glomerular function that precede proteinuria and other signs of renal dysfunction.

Previous studies indicated that P_{alb} increases within one hour after TBI [12]. Present results confirmed these findings and show that increased P_{alb} can be observed as early as 15 minutes post-TBI (Figure 1B) and remains increased at all time points studied up to 24 hours post-TBI. This finding suggests that increased P_{alb} is an immediate consequence of the subcellular changes caused by TBI. Another immediate consequence of radiation injury was reflected in altered glomerular ultrastructure (Figure 2). The glomerular permeability barrier is constituted by the capillary cells, the basement membrane and the visceral epithelial cells (podocytes). Foot processes from podocytes interdigitate around the capillary loops and form slit-pore junctions. Changes in podocyte morphology and in capillary diameter each confirm early abnormalities in glomerular structure after irradiation. Previously, we have determined that isolation procedures do not cause significant changes in glomerular structure [24]. Detailed studies will be required to determine the significance of these observations, and that of the likely changes in tubular and interstitial structures, in the progression and manifestation of late effects of radiation injury.

There are no proven molecular markers of the initial renal injury caused by radiation. Urinary proteins may originate from filtration of plasma proteins, tubular protein leakage or secretion, the presence of sloughed cells, detachment of membrane surface-anchored proteins or membrane vesicle/exosome excretion [25,26]. TBI may cause passage of proteins into the tubular lumen and excretion of proteins that are normally absent in urine. Membrane proteins, cell surface or intracellular enzymes, junctional proteins of podocytes or tubules are likely candidate proteins. There is increasing interest in the application of biofluids, including urine, to identify markers of pathophysiological changes in a large number of diseases [27].

We hypothesize that there will be detectable changes in the urine proteome following irradiation that can be detected by a high throughput proteomic screen. To test this, we have analyzed urine from irradiated and age matched control animals using LC-MS/MS methods. The resulting mass spectra were searched against a rodent proteomic database and the high confidence hits determined. In addition to identifying plasma and renal proteins that changed in level in response to irradiation, we were able to detect radiation induced protein modifications. Two-dimensional gel electrophoresis and the initial analysis of the MS/MS results showed increased number of proteins. In order to identify groups of proteins based on cellular compartment, molecular function and biological process we analyzed MS/MS results using Gene Ontology (GO) algorithm (Figure 4 A-C). MS/MS spectra were searched with a two pass search strategy that uses the results of a simple search to conduct a more detailed search. The irradiated and control groups were compared with regard to the numbers of proteins under various categories. GO analysis based on cellular compartment indicated that proteins from the membrane, extracellular region, nucleus, cytoplasm and cytoskeleton compartments accounted for most of the protein count. Presence of many proteins from the membrane compartment is consistent with them having been shed into the urine by the kidney or being transferred from the plasma. Nuclear or cytoplasmic proteins indicate certain degree of cell lysis (Figure 4A). GO analysis based on functional classes indicated glycosylphosphatidylinositol (GPI) anchor binding, calmodulin binding, sugar binding, serine-type endopeptidase activity, protein dimerization, kinases activity, motor activity, structural constituents of the cytoskeleton, peptidase activity, nucleotide binding, ATP binding and calcium binding classes of proteins (Figure 4B). Of these proteases and protease inhibitors are of special significance because they are involved in tubular function and, response to injury by circulating cells and the complement system. GO analysis based on biological process indicated increased numbers of proteins associated with all biological processes examined following TBI. Proteins associated with anti-apoptosis, muscle contraction and epidermis growth groups showed increased levels in the TBI group. Proteins involved in lipid metabolism, oxidative stress responses, anti-inflammation responses, cell proliferation, cytoskeletal organization and proteolysis groups also increased in number. (Figure 4C).

Urinary levels of several proteinases and proteinase inhibitors were altered by total body irradiation (Figure 5A and 5B). Most noticeable increase occurred in the levels of Kallikrein 1-related peptidase b24 (chymotrypsin-like activity) precursor (Glandular kallikrein K24, Tissue kallikrein 24, gi|48428341). Figure 5B shows that this was paralleled by a decrease in the levels of serine protease inhibitor A3K precursor (Serpine A3K, kallikrein-binding protein, gi|266407). We also found that products of chymotrypsin-like activity are detectable present in the 24 hour urine after TBI (Results not shown). Changes in kallikrein-kinin system proteins may influence vascular function, coagulation and inflammation processes. Plasma and tissue kallikreins act on precursor kininogens to synthesize vasodilatory bradykinin or kallidin peptides.

Carboxypeptidases, neutral endopeptidase or angiotensin converting enzyme are proteinases that degrade and inactivate bradykinin and kallidin. Increased urinary kallikrein and inhibitor are biologically significant and may be valuable in understanding the mechanism of development of radiation nephropathy [28,29].

Finally, GO analysis suggested increased urinary proteins involved in several biological processes including anti-apoptotic, muscle contraction, cell proliferation, cell differentiation, cytoskeletal organization, intracellular signaling, epidermis development, lipid metabolism, proteolysis, oxidative stress, inflammation (Figure 4C). Many of the changes in these pathways could result from the radiation-induced generation of oxidant species.

As described above, we used a two pass approach to search spectra with unconstrained (non-tryptic) protease specificity for amino acid oxidation as a post-translational modification (PTM). PTMs contribute to a large number of regulatory and signaling events in the cell. A number of protein modifications are known including, of proteins due to phosphorylation, methylation, nitration, acetylation, deamination, alkylation, cysteine thiol oxidation, carbonylation. We have shown that phosphorylation of glomerular proteins plays a role in the regulation of P_{alb} [30] and methylated arginines (ADMA, L-NMMA) increase P_{alb} through inhibition of nitric oxide synthase activity [31]. Presently, we focused on the oxidative changes that might occur following total body X-irradiation.

Ionizing radiation causes oxidative damage and chemical changes in DNA, lipids and proteins. We speculated that TBI-induced injury may result in excretion of modified proteins in the urine [32,33]. Since both rats and human kidneys are sensitive to renal radiation injury [34], the nature of proteins excreted in urine after radiation may be comparable. Our results of all the amino acids that are likely to generate oxidation products only oxidized histidine showed a 3-fold increase. Selective oxidation of histidine as an early effect of irradiation has significant implications. Histidine is an essential amino acid for humans and rats and its deficiency affects whole-body protein turnover rate and levels of hemoglobin, albumin and transferrin [35]. The neutral pK_a of its imidazole side chain allows participation in acid/base catalysis and proton transfer reactions at physiological pH. The nucleophilicity of imidazole also makes it a good ligand for electrophilic metal ions, such as zinc in many enzymes such as carboxypeptidase. In the hemoglobin molecule, one histidine stabilizes the porphyrin structure through a covalent linkage with the heme iron. Another histidine residue prevents oxidation of iron by oxidizing agents and blocks carbon monoxide from binding to the heme iron while allowing oxygen to bind easily. Oxidative damage to protein histidine by hydroxyl radicals or products of lipid oxidation such as HNE (4-hydroxynon-2-enal) has been reported [36,37]. Therefore, radiation-induced increase in oxidized histidine is likely to affect acid/base balance by enzymes (e.g. carbonic anhydrase) and oxygen transport by hemoglobin.

Present results indicate a large increase in the number of urinary proteins without a detectable increase in total urinary protein following TBI. This anomaly led us to analyze our MS/MS results for plasma proteins. Results showed a decrease in the urinary levels of albumin and a smaller decrease in immunoglobulins. One of the several contributing factors to this observation could be a transient increase in the proximal tubular reuptake of albumin. Ionizing radiation causes injury in a tissue specific manner and some tissues are more sensitive than others. We have found that such differences in radiosensitivity may exist within the same organ such as the kidneys. We have previously reported that while increased glomerular protein permeability was detectable within one hour after irradiation the tubular reuptake of filtered albumin appeared to decline over a longer period suggesting different responses of glomeruli and tubules to ionizing radiation [38]. Evidence from other studies also suggests differences in tubular and glomerular response to injury. Recently we used intravital two photon microscopy to observe the effect of puromycin aminonucleoside (PAN) on glomerular albumin permeability and tubular reuptake of injected fluorescence tagged albumin. Results showed that PAN injection resulted in increased glomerular albumin permeability within an hour and proteinuria after 5 days. We also noted increased fluorescence in tubular cells and lumina by 3 days after PAN and development of proteinuria after intravenously injection of PAN [39]. However, the molecular mechanisms responsible for the development of renal injury leading to organ dysfunction are not well understood. The specific chain of events that leads to increased urinary proteins following TBI needs to be investigated. In contrast to albumin and immunoglobulin, urinary levels of alpha-1-acid glycoprotein (orosomucoid) were increased. Increased urinary alpha-1-acid glycoprotein has previously been associated with trauma, stress and type II diabetes [40,41]. Changes in urinary albumin imply a number of physiological

responses that are likely set off by irradiation. Detailed studies will be required to understand the significance and mechanism of these changes.

5 Concluding Remarks

These studies provide novel initial data on the early effects of total body irradiation in a rat model, and suggest that the urine proteome could serve as a early biomarker of radiation injury. TBI alters glomerular ultrastructure and increases glomerular permeability to albumin *in vitro* suggesting injury to the glomerular filtration barrier *per se*. MS/MS analysis of the urine proteome suggests greater than two-fold changes in the urinary levels of selected plasma and renal proteins. Increased serine proteinases of the complement pathway and oxidized histidine represent systemic changes. Increased tissue kallikrein and membrane associated peptidases accompanied by a parallel decrease in several proteinase inhibitors indicate vascular changes and renal injury. Decreased urinary albumin is suggestive of the net outcome of a number of changes in renal physiology. Together, the urinary protein profile reflects the complexity of systemic and renal changes within one day after TBI. Studies of the urine proteome may be useful in identifying the early biomarkers of radiation injury.

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a= initial diameter in 5% BSA

b= final diameter in 1% BSA

Figure 1a. A. Glomerular albumin permeability is determined by measuring changes in glomerular volume in response to an experimental oncotic gradient

A glomerulus is held in medium containing 5% BSA and images are recorded using videomicroscopy. Bath medium is changed to one containing 1% BSA and a second image is obtained. Four glomerular diameters in 5% BSA (a) and 1% BSA (b) media are measured at 45° angles and the geometric mean used to calculate glomerular volume in each condition. Reflection coefficient, σ , is calculated from the ratio of average volume change of experimental and control glomeruli and is used to calculate P_{alb} ($1-\sigma$) as described in Methods.

Figure 1b. B. Total body irradiation (TBI) increases glomerular albumin permeability (P_{alb})
 P_{alb} was determined in glomeruli isolated at 15, 30, 60 min and 24 hours after TBI (10 Gy) or sham-treatment (control). TBI resulted in increased P_{alb} within 15 minutes and at all other time points studied ($P < 0.001$ vs. control, $n=15$ glomeruli from 3 rats).

Figure 2. Scanning Electron Microscopy (SEM) suggests altered glomerular ultrastructure within 24 hour after TBI

Glomeruli were isolated from sham-treated controls and irradiated rats at 24 hours after TBI. The urinary aspects of representative glomeruli from the two groups are shown. Capillary width is noticeably decreased in the irradiated group. Podocyte cell body (CB, arrowheads) and the primary processes from cell bodies appeared collapsed. Tight interdigitations formed by foot processes are partially lost (areas marked by circles) and in some areas exposed basement membrane can be observed.

Figure 3. Two- Dimensional Electrophoresis shows changes in the urinary protein profile within 24 hours after TBI

Urinary samples from the sham-control and TBI groups were desalted by membrane filtration. Samples were fractionated in the first dimension by isoelectric focusing using IPG strips followed by a second dimension size-based separation using 4-20% SDS polyacrylamide gel. Protein spots were visualized by silver staining. An altered protein profile is indicated by the higher number of proteins in the irradiated group.

Figure 4a. A. Gene Ontology analysis suggests varying effects of TBI on cellular components
Protein assignments based on MS/MS results were analyzed to determine the changes caused in proteins grouped by molecular function induced by TBI. For each of the GO cellular component categories, we determined the number of observed proteins assigned to it. We compared the irradiated and control groups with regard to the numbers of proteins showing increases, decreases or no change in each GO cellular component. Results of analysis for 25 cellular components groups indicate that keratin and intermediate filament proteins increased in the irradiated group. Increases (and decreases) in proteins of the membrane, nuclear, cytoskeleton and extracellular regions were the most numerous. Membrane proteins from all components combined constituted the single largest group of proteins that increased.

Figure 4b. B. Gene Ontology analysis of MS/MS results suggests distinct effects of TBI on different functional classes of proteins

Protein assignments based on MS/MS results were analyzed to determine the changes caused in proteins grouped by molecular function induced by TBI. For each of the GO molecular function categories, we determined the number of observed proteins assigned to it. We compared the irradiated and control groups with regard to the numbers of proteins showing increases, decreases or no change in each GO molecular function category. GPI (glycosylphosphatidylinositol) anchor binding proteins increased in the urine from TBI rats. Proteins that bind to metal ions, nucleic acids, sugars, ATP or cytoskeleton constituents were most frequently observed to increase followed by hydrolysis (peptidases, hydrolases etc.)-related proteins..

Figure 4c. C. Gene Ontology analysis of MS/MS results suggests distinct effects of TBI on biological processes

Protein assignments based on MS/MS results were analyzed to determine the radiation-induced changes in proteins grouped under biological processes. For each of the GO biological process categories, we determined the number of observed proteins assigned to it. We compared the irradiated and control groups with regard to the numbers of proteins showing increases, decreases or no change in each GO biological process category.. TBI resulted in increased numbers of proteins associated with all biological processes examined. Proteins associated with anti-apoptosis, muscle contraction and epidermis growth groups showed increased levels in the TBI group. Proteins involved in lipid metabolism, oxidative stress responses, anti-inflammation responses, cell proliferation, cytoskeletal organization and proteolysis groups also increased in number.

Figure 5a. A. TBI alters urinary profile of proteinases

Protein assignments based on MS/MS results were analyzed to determine the changes caused in specific proteases induced by TBI. Scan counts correspond to the number of spectra that were assigned to peptides originating from each of the proteins. For this analysis, trypsin was not included since trypsin was used during sample preparation for LC-MS/MS. K1B24 (Kallikrein 1-related peptidase b24 precursor, Glandular kallikrein K24, Tissue kallikrein 24 or mGK-24, gi 48428341), a serine proteinase, was found to be present only in the TBI group. Fibronectin was detected under this category of proteins although it is a matrix protein.

Key to protein names: ACD10_MOUSE Acyl-CoA dehydrogenase family member 10; CATC_RAT Dipeptidyl-peptidase 1; TMM27_RAT Collectrin / Transmembrane protein 27, SNARE complex regulator; **A4_RAT Amyloid beta A4 protein**; LGMN_RAT Legumain / Asparaginyl endopeptidase; NEP_RAT Neprilysin; DPP4_RAT Dipeptidyl peptidase 4; RISC_RAT Retinoid-inducible serine carboxypeptidase; DPP2_RAT Dipeptidyl-peptidase 2; EPCR_RAT Endothelial protein C receptor; KLK8_RAT Prostatic glandular kallikrein-8 / Tissue kallikrein; DYH8_MOUSE Ciliary dynein heavy chain 8; CFAD_RAT Complement factor D / C1 of the classical pathway; ATS20_MOUSE ADAMTS-20 / A disintegrin and metalloproteinase with thrombospondin motifs 20; PLMN_RAT Plasminogen; MEP1B_RAT Meprin A subunit beta / Endopeptidase2; KLK7_RAT Glandular kallikrein-7, submandibular/renal; KLK10_RAT Glandular kallikrein-10, submandibular/renal; CFAB_MOUSE Complement factor B / C3/C5 convertase; **KNT1_RAT T-kininogen 1** / Major acute phase protein [MAP]; AMPN_RAT **Aminopeptidase N** / Kidney Zn peptidase; GGH_RAT Gamma-glutamyl hydrolase; CATB_RAT Cathepsin B; MEP1A_RAT Meprin A subunit alpha / Endopeptidase 2; FINC_RAT Fibronectin; K1B24_MOUSE Kallikrein 1-related peptidase b24 /Tissue kallikrein 24.

Figure 5b. B. TBI causes changes in the urinary proteinase inhibitors

Protein assignments based on MS/MS results were analyzed to determine the changes caused in proteinase inhibitors induced by TBI. Scan counts correspond to the number of spectra that were assigned to peptides originating from each of the proteins. SPA3K (Serine protease inhibitor A3K precursor, Kallikrein-binding protein, gi 266407) was observed to be the most abundant proteinase inhibitor in the urine. Irradiation produced a greater than 50% decrease in urinary SPA3K compared to the control group.

Key to protein names: **PEBP1_RAT** Phosphatidylethanolamine-binding protein 1; **A4_RAT** Amyloid beta A4 protein; **A1I3_RAT** Alpha-1-inhibitor 3; **MUG2/RAT** Murinoglobulin-2; **A1M_RAT** Alpha-1-macroglobulin; **MUG1_RAT** Murinoglobulin-1; **SPA3N_RAT** Serine protease inhibitor A3N; **ANGT_RAT** Angiotensinogen; **CO4_RAT** Complement C4; **KNT2_RAT** T-kininogen 2; **KNT1_RAT** T-kininogen 1; **FETUA_RAT** Alpha-2-HS-glycoprotein / Fetuin A; **PI16_MOUSE** Peptidase inhibitor 16 / Cysteine-rich protease inhibitor; **CBG_RAT** Corticosteroid-binding globulin; **A1AT_RAT** Alpha-1-antiproteinase; **CYTC_RAT** Cystatin C. Inhibitor of cysteine proteinases, known local regulator of cathepsin B, H, and L; **SPI21_RAT** Serine protease inhibitor 2.1; **AMBP_RAT** AMBP protein / AMBP protein; **SPA3L_RAT** Serine protease inhibitor A3L; **SPA3K_RAT** Serine protease inhibitor A3K.

Figure 6. TBI causes decreases in urinary albumin and immunoglobulins

MS/MS results were further analyzed for plasma proteins. Decreased urinary albumin and immunoglobulin and, increased alpha-1-acid-glycoproteins were observed at 24 hours after TBI.

Table 2
Modified amino acids detected by LC-MS/MS analysis

Spectra from irradiated and control urine samples were searched with Sequest with the following dynamic modifications: M +16 (oxidized methionine), P + 16 (oxidized proline), H +16 (oxidized histidine), K -1 (oxidized lysine) and R -43 (arginine glutamic semialdehyde). The number of spectra are the number of spectra containing the modification with probability scores of >0.6 assigned to proteins with probability scores of >0.9. Total Spectra is the total number spectra that met the above criteria for each sample.

Modification	Irradiated (spectra)	% Total	Control (spectra)	% Total
Methionine Oxidation	949	2.58%	1199	4.72%
Proline Oxidation	306	0.83%	197	0.78%
Histidine Oxidation	229	0.62%	53	0.21%
Lysine Oxidation	307	0.83%	297	1.17%
Arginine glutamic semialdehyde	421	1.14%	315	1.24%
Total Spectra	36,787		25,386	

Table 1

Protein	Accession	Description	Scan Count	Irradiated	Control Ratio
K1B24 MOUSE	Q61754	Kallikrein 1-related peptidase b24 precursor (EC 3.4.21.35) (Glandular kallikrein K24) (Tissue kallikrein 24) (mGK-24).	653	653	NA
OCAL1 MOUSE	Q60847	Collagen alpha-1(XII) chain precursor.	145	145	NA
OOSTP RAT	P08721	Osteopontin precursor (Bone sialoprotein-1) (Secreted phosphoprotein1) (SPP-1).	64	64	NA
MPDZ RAT	O55164	Multiple PDZ domain protein (Multi-PDZ domain protein 1).	45	45	NA
AMPN RAT	P15684	Aminopeptidase N (EC 3.4.11.2) (rAPN) (Alanyl aminopeptidase) (Microsomal aminopeptidase) (Aminopeptidase M) (APM) (Kidney Znpeptidase) (KZP) (CD13 antigen).	32	32	NA
K2C1 RAT	Q61MF3	Keratin, type II cytoskeletal 1 (Cytokeratin-1) (CK-1) (Keratin-1) (Type II keratin Kb1).	31	31	NA
SNX13 MOUSE	Q6PHS6	Sorting nexin-13.	27	27	NA
GALT3 MOUSE	P70419	Polypeptide N-acetylgalactosaminyltransferase 3 (EC 2.4.1.41) (Protein-UDP acetylglucosaminyltransferase 3) (UDP-GalNAc: polyglutamate N-acetylgalactosaminyltransferase 3) (Polypeptide GalNAc transferase 3) (GalNAc-T3) (pp-GalNTase 3).	25	25	NA
K2C8 MOUSE	P11679	Keratin, type II cytoskeletal 8 (Cytokeratin-8) (CK-8) (Keratin-8) (K8) (Cytokeratin endo A).	24	24	NA
ACTB RAT	P60711	Actin, cytoplasmic 1 (Beta-actin).	23	23	NA
ACTG RAT	P63259	Actin, cytoplasmic 2 (Gamma-actin).	23	23	NA
DEK RAT	Q6AXS3	Protein DEK.	22	22	NA
ABCC9 RAT	Q63563	ATP-binding cassette transporter sub-family C member 9 (Sulfonylurea receptor 2).	21	21	NA
ZEPI MOUSE	Q03172	Zinc finger protein 40 (Transcription factor alphaA-CRYBP1) (Alpha A-crystallin-binding protein 1) (Alpha A-CRYBP1).	20	20	NA
ACTH RAT	P63269	Actin, gamma-meric smooth muscle (Smooth muscle gamma actin) (Gamma-2-actin) (Alpha-actin-3).	20	20	NA
ACTA RAT	P62738	Actin, aortic smooth muscle (Alpha-actin-2).	20	20	NA
LRP2 RAT	P98158	Low-density lipoprotein receptor-related protein 2 precursor (Megalin) (Glycoprotein 330) (gp330).	20	20	NA
ACTS RAT	P68136	Actin, alpha skeletal muscle (Alpha-actin-1).	19	19	NA
ACTC RAT	P68035	Actin, alpha cardiac muscle 1 (Alpha-cardiac actin).	19	19	NA
KADI1 RAT	P39069	Adenylate kinase isoenzyme 1 (EC 2.7.4.3) (ATP-AMP transphosphorylase) (AK1) (Mvokinase).	19	19	NA
K1C10 RAT	Q6FW6	Keratin, type I cytoskeletal 10 (Cytokeratin-10) (CK-10) (Keratin-10) (K10) (Type I keratin K410).	17	17	NA
CFAB MOUSE	P04186	Complement factor B precursor (EC 3.4.21.47) (C3/C5 convertase) (Contains: Complement factor B Ba fragment; Complement factor B Bb fragment).	15	15	NA
PRAX MOUSE	Q55103	Periaxin.	14	14	NA
ELP4 MOUSE	Q9ER73	Elongator complex protein 4 (PAX6 neighbor gene protein).	14	14	NA
MEPB RAT	P28826	Meprin A subunit beta precursor (EC 3.4.24.18) (Endopeptidase-2).	13	13	NA
UK114 RAT	P52759	Ribonuclease UK114 (EC 3.1.1.1) (14.5 kDa translational inhibitor protein) (Perchloric acid soluble protein).	13	13	NA
THIO RAT	P11232	Thioredoxin (Trx).	13	13	NA
KLK7 RAT	P36373	Glandular kallikrein n=7, submandibular/renal precursor (EC 3.4.21.35) (Tissue kallikrein) (RGK-7) (RSKG-7) (Kallikrein-related protein K1) (Esterase B) (Proteinase A).	13	13	NA
SLC31 RAT	Q64319	Neutral and basic amino acid transport protein rBAT (B(0,+)-type amino acid transport protein) (NAA-TR) (D2).	12	12	NA
AT20 MOUSE	P59511	ADAMTS-20 precursor (EC 3.4.24.-) (A disintegrin and metalloproteinase with thrombospondin motifs 20) (ADAM-TS 20) (ADAM-TS20).	11	11	NA
SON MOUSE	Q9QX47	SON protein.	11	11	NA
SPA3N RAT	P09006	Serine protease inhibitor A3N precursor (Serpin A3N) (Contrapsin-like protease inhibitor 6) (CPI-26) (Serine protease inhibitor 3) (SPI-3) (SPI-2.2).	11	11	NA
PLMN RAT	Q01177	Plasminogen precursor (EC 3.4.21.7) (Contains: Plasmin heavy chain A; Activation peptide; Angiostatin; Plasmin heavy chain A, short form; Plasmin light chain B).	11	11	NA
MYPT1 MOUSE	Q9DBR7	Protein phosphatase 1 regulatory subunit 12A (Myosin phosphatase-targeting subunit 1) (Myosin phosphatase target subunit 1).	11	11	NA
ESCO1 MOUSE	Q69269	N-acetyltransferase ESCO1 (EC 2.3.1.-) (Establishment of cohesion 1 homolog 1) (ECOI homolog 1).	10	10	NA
REG3G RAT	P42854	Regenerating islet-derived protein 3 gamma a precursor (Reg III-gamma) (Pancreatitis-associated protein 3).	9	9	NA
MIF RAT	P30904	Macrophage migration inhibitory factor (MIF) (Phenylpyruvate tautomerase) (EC 5.3.2.1) (Glutathione-binding 13 kDa protein).	8	8	NA
SVIL MOUSE	Q8K4L3	Supervillin (Archvillin) (p205/p250).	8	8	NA
DYH8 MOUSE	Q91X00	Ciliary dynein heavy chain 8 (Axonemal beta dynein heavy chain 8).	8	8	NA
DNL1 RAT	Q9JHY8	DNA ligase 1 (EC 6.5.1.1) (DNA ligase 1) (Polydeoxyribonucleotidesynthase [ATP] 1).	8	8	NA
HYAL1 RAT	Q76HNI	Hyaluronidase-1 precursor (EC 3.2.1.35) (Hyal-1) (Hyaluronoglucosaminidase-1).	8	8	NA
NRIP1 MOUSE	Q8CBD1	Nuclear receptor-interacting protein 1 (Nuclear factor R1P140) (Receptor-interacting protein 140).	7	7	NA
KLK8 RAT	P36374	Prostatic glandular kallikrein-8 precursor (EC 3.4.21.35) (Tissue kallikrein) (PI kallikrein) (RGK-8).	7	7	NA
SPTB2 MOUSE	Q62261	Spectrin beta chain, brain 1 (Spectrin, non-erythroid beta chain 1) (Beta-II spectrin) (Fodrin beta chain) (Embryonic liver fodrin).	7	7	NA
MYO16 RAT	Q9ERC1	Myosin-XVI (Unconventional mvosin-16) (Myosin heavy chain mvr 8).	7	7	NA

Protein	Accession	Description	Scan Count	Irradiated	Control	Ratio
K2C1B_RAT	Q6IG01	Keratin, type II cytoskeletal 1b (Keratin-77) (Type II keratin Kb39).	7	7	7	NA
EPGR_RAT	Q4V8II	Endothelial protein C receptor precursor (Endothelial cell protein C receptor) (Activated protein C receptor) (APC receptor).	7	7	7	NA
CO2A1_RAT	P05539	Collagen alpha-1(I) chain precursor (Alpha-1 type II collagen) (Contains: Chondrocalcin).	6	6	6	NA
CT152_MOUSE	Q9D5U8	Uncharacterized protein C20orf152 homolog.	6	6	6	NA
RUSC1_MOUSE	Q8BG26	RUN and SH3 domain-containing protein 1.	6	6	6	NA
CYFP1_MOUSE	Q7TMB8	Cytoplasmic FMR1-interacting protein 1 (Specifically Rac1-associated protein 1) (Sra-1).	5	5	5	NA
DP22_RAT	Q9EPB1	Dipeptidyl-peptidase 2 precursor (EC 3.4.14.2) (Dipeptidyl-peptidase II) (DPP II) (Dipeptidyl aminopeptidase II) (Quiescent cell prolinedipeptidase) (Dipeptidyl peptidase 7).	5	5	5	NA
KV1A1_MOUSE	P01632	Ig kappa chain V-1 region S107A.	5	5	5	NA
UBQL1_RAT	Q9JJP9	Ubiquitin-1 (Protein linking IAP with cytoskeleton 1) (PLIC-1).	5	5	5	NA
RL28_RAT	P17702	60S ribosomal protein L28.	5	5	5	NA
CC130_RAT	Q32PZ9	Coiled-coil domain-containing protein 130.	5	5	5	NA
VLDLR_RAT	P98166	Very low-density lipoprotein receptor precursor (VLDL receptor) (VLDL-R).	5	5	5	NA
PDZD1_RAT	Q9JJ40	PDZ domain-containing protein 1 (Na/Pi cotransporter C-terminal-associated protein) (NaPi-Cap1) (Na(+)/H(+) exchanger regulatory factor 3) (Sodium-hydrogen exchanger regulatory factor 3) (Dietary Pi-regulated RNA-1) (Diphospho-1) (C-terminal-linking and modulating protein).	5	5	5	NA
TAF6_RAT	Q63801	Transcription initiation factor TFIID subunit 6 (Transcription initiation factor TFIID 70 kDa subunit) (TAF(II)70) (TAFII-80) (TAFII80) (p80).	5	5	5	NA
SAMP_RAT	P23680	Serum amyloid P-component precursor (SAP).	5	5	5	NA
K22E_RAT	Q6IG02	Keratin, type II cytoskeletal 2 epidermal (Cytokeratin-2e) (K2e) (CK2e) (keratin-2) (Type II keratin Kb2).	5	5	5	NA
DGKG_MOUSE	Q9JWG7	Diacylglycerol kinase gamma (EC 2.7.1.107) (Diglyceride kinase gamma) (DGK-gamma) (DAG kinase gamma) (88 kDa diacylglycerol kinase).	5	5	5	NA
K0423_MOUSE	Q6A070	Uncharacterized protein KIAA0423.	4	4	4	NA
S10A6_RAT	P05964	Protein S100-A6 (S100 calcium-binding protein A6) (Calcyclin) (Prolactin receptor-associated protein).	4	4	4	NA
HYOU1_RAT	Q63617	Hypoxia up-regulated protein 1 precursor (150 kDa oxygen-regulated protein) (Orp150).	4	4	4	NA
HS90A_CRIGR	P46633	Heat shock protein HSP 90-alpha (HSP 86).	4	4	4	NA
WDR5_RAT	Q498M4	WD repeat protein 5.	4	4	4	NA
CD59_RAT	P27274	CD59 glycoprotein precursor (Membrane attack complex inhibition factor) (MACIF) (MAC-inhibitory protein) (MAC-IP) (Protectin).	4	4	4	NA
K1C14_RAT	Q6JFV1	Keratin, type I cytoskeletal 14 (Cytokeratin-14) (CK-14) (Keratin-14) (K14) (Type I keratin Ka14).	4	4	4	NA
BIR1E_MOUSE	Q9R016	Baculoviral IAP repeat-containing protein 1e (Neuronal apoptosis-inhibitory protein 5).	4	4	4	NA
GPR98_MOUSE	Q8VHN7	G-protein coupled receptor 98 precursor (Monogenic audiogenic seizure susceptibility protein 1) (Very large G-protein coupled receptor 1) (Neurepin).	4	4	4	NA
JIP1_RAT	Q9R237	C-jun-amino-terminal kinase-interacting protein 1 (JNK-interacting protein 1) (JIP-1) (JNK MAP kinase scaffold protein 1) (Islet-brain-1) (IB-1) (Mitogen-activated protein kinase 8-interacting protein 1) (JIP-1-related protein) (JRP).	4	4	4	NA
ALPK2_MOUSE	Q9JZB0	Alpha-protein kinase 2 (EC 2.7.11.-) (Heart alpha-protein kinase).	4	4	4	NA
MYOF_MOUSE	Q69ZN7	Myoferlin (Fer-1-like protein 3).	4	4	4	NA
CCNL2_RAT	Q510H5	Cyclin-L2.	4	4	4	NA
CP2DR_MOUSE	Q9QYGG	Cytochrome P450 2D27 (EC 1.14.14.-) (CYP2D7).	3	3	3	NA
TRY4_RAT	P12788	Trypsin-4 precursor (EC 3.4.21.4) (Trypsin IV) (Pretirpsinogen IV).	3	3	3	NA
PIPA_RAT	P10111	Peptidyl-prolyl cis-trans isomerase A (EC 5.2.1.8) (PPIase A) (Rotamase A) (Cyclophilin A) (Cyclosporin A-binding protein) (p31) (p1B15).	3	3	3	NA
K2C4_RAT	Q6IG00	Keratin, type II cytoskeletal 4 (Cytokeratin-4) (CK-4) (Keratin-4) (K4) (Type II keratin Kb4).	3	3	3	NA
FAT2_RAT	Q88277	Protocadherin Fat 2 precursor (Multiple epidermal growth factor-like domains 1).	3	3	3	NA
ITSN1_RAT	Q9WVE9	Intersectin-1 (EH domain and SH3 domain regulator of endocytosis 1).	3	3	3	NA
MYH3_RAT	P12847	Myosin-3 (Myosin heavy chain 3) (Myosin heavy chain, fast skeletal muscle, embryonic).	3	3	3	NA
K1C15_RAT	Q6JFV3	Keratin, type I cytoskeletal 15 (Cytokeratin-15) (CK-15) (Keratin-15) (K15) (Type I keratin Ka15).	3	3	3	NA
FBX41_MOUSE	Q6NS60	F-box only protein 41.	3	3	3	NA
MUG2_RAT	Q6IE52	Murinoglobulin-2 precursor.	3	3	3	NA
TDRD6_MOUSE	P61407	Tudor domain-containing protein 6.	3	3	3	NA
SRM2_MOUSE	Q8BT18	Serine/arginine repetitive matrix protein 2.	3	3	3	NA
LYSCP_MOUSE	P17897	Lysozyme C type P precursor (EC 3.2.1.17) (1,4-beta-N-acetylmuramidase C).	3	3	3	NA
PCOC1_RAT	Q08628	Procollagen C-endopeptidase enhancer 1 precursor (Procollagen COOH-terminal proteinase enhancer 1) (Procollagen C-proteinase enhancer 1) (PCPE-1) (Type I procollagen COOH-terminal proteinase enhancer) (Type I procollagen C-proteinase enhancer protein).	3	3	3	NA
TRRAP_MOUSE	Q80YV3	Transformation/transcription domain-associated protein (Tra1 homolog).	3	3	3	NA
CPT2_MOUSE	P52825	Carnitine O-palmitoyltransferase 2, mitochondrial precursor (EC 2.3.1.21) (Carnitine palmitoyltransferase II) (CPT II).	3	3	3	NA

Protein	Accession	Description	Scan Count	Irradiated	Control	Ratio
NEP_RAT	P07861	Neprilysin (EC 3.4.24.11) (Neutral endopeptidase) (NEP) (Enkephalinase) (Neutral endopeptidase 24.11) (Atriopeptidase) (CD10 antigen).		3	3	NA
		Dipeptidyl peptidase 4 (EC 3.4.14.5) (Dipeptidyl peptidase IV) (DPPIV) (T-cell activation antigen CD26) (GP110 glycoprotein) (Bilecanalculus domain-specific membrane glycoprotein) [Contains:Dipeptidyl peptidase 4 membrane form (Dipeptidyl peptidase IV membrane form); Dipeptidyl peptidase 4 soluble form (Dipeptidyl peptidase IV soluble form); Dipeptidyl peptidase 4 60 kDa soluble form (Dipeptidyl peptidase IV 60 kDa soluble form)].				
DPP4_RAT	P14740			3	3	NA
HRX_MOUSE	P55200	Zinc finger protein HRX (ALL-1).		3	3	NA
MAP4_MOUSE	P27546	Microtubule-associated protein 4 (MAP-4).		3	3	NA
SMCA5_MOUSE	P0912W3	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A member 5 (EC 3.6.1.1-) (Sucrose nonfermenting protein 2 homolog) (mSnf2h).		3	3	NA
ITB2L_MOUSE	Q3UW74	Integrin beta-2-like protein precursor (Protein pactolus).		3	3	NA
COIA2_RAT	P02466	Collagen alpha-2(I) chain precursor (Alpha-2 type I collagen).		3	3	NA
CPSM_RAT	P07756	Carbamoyl-phosphate synthase [ammonial, mitochondrial] precursor (EC 6.3.4.16) (Carbamoyl-phosphate synthetase I) (CPSase I).		2	2	NA
GBP2_RAT	O63663	Interferon-induced guanylate-binding protein 2 (GTP-binding protein 2) (Guanine nucleotide-binding protein 2) (GBP-2) (p67).		2	2	NA
LMAN2_MOUSE	Q09DBH5	Vesicular integral-membrane protein VIP36 precursor (Lectin, mannose-binding 2).		2	2	NA
PDZD2_RAT	Q9QZR8	PDZ domain-containing protein 2 (PDZ domain-containing protein 3) (Plakophilin-related armadillo repeat protein-interacting PDZ protein).		2	2	NA
A113_RAT	P14046	Alpha-1-inhibitor 3 precursor (Alpha-1-inhibitor III) (Alpha-1-inhibitor 3 variant II).		2	2	NA
GALT5_RAT	O88422	Polypeptide N-acetylgalactosaminyltransferase 5 (EC 2.4.1.41) (Protein-UDP acetylgalactosaminyltransferase 5) (UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferase 5) (PolypeptideGalNAc transferase 5) (GalNAc-T5) (pp-GalNTase 5).		2	2	NA
CALM_RAT	P62161	Calmodulin (CaM).		2	2	NA
STAB1_MOUSE	Q8R4Y4	Stabilin-1 precursor (Fascilin, EGF-like, laminin-type EGF-like and link domain-containing scavenger receptor 1) (FEEL-1).		2	2	NA
CNTN6_MOUSE	Q9JMB8	Contactin-6 precursor (Neural recognition molecule NB-3) (mNB-3).		2	2	NA
LGMN_RAT	Q9R0J8	Legumain precursor (EC 3.4.22.34) (Asparaginyl endopeptidase) (Protease, cysteine 1).		2	2	NA
DIAC_RAT	O01460	Di-N-acetylchitobiase precursor (EC 3.2.1.-).		2	2	NA
ZFP62_MOUSE	Q8C827	Zinc finger protein 62 (Zfp-62) (ZT3).		2	2	NA
JAK2_MOUSE	Q62120	Tyrosine-protein kinase JAK2 (EC 2.7.10.2) (Janus kinase 2) (JAK-2).		2	2	NA
GSN0_RAT	P48508	Glutamate-cysteine ligase regulatory subunit (EC 6.3.2.2) (Gamma-glutamylcysteine synthetase) (Gamma-ECS) (GCS light chain) (Glutamate-cysteine ligase modifier subunit).		2	2	NA
BCL9_MOUSE	Q9D219	B-cell lymphoma 9 protein (Bcl-9).		2	2	NA
MYH7_RAT	P02564	Myosin-7 (Myosin heavy chain 7) (Myosin heavy chain, cardiac muscle beta isoform) (MyHC-beta) (Myosin heavy chain slow isoform) (MyHC-slow).		2	2	NA
USH2A_MOUSE	Q2QJ47	Usher precursor (Usher syndrome type-2 A protein homolog) (Ushersyndrome type IIa protein homolog).		2	2	NA
TMM27_RAT	Q9ESG3	Collectrin precursor (Transmembrane protein 27).		2	2	NA
CAD23_RAT	P58365	Cadherin-23 precursor (Otocadherin).		2	2	NA
DBF4_MOUSE	Q9QZ41	Protein DBF4 homolog (MuDBF4).		2	2	NA
ITPR2_MOUSE	Q9Z329	Inositol 1,4,5-trisphosphate receptor type 2 (Type 2 inositol 1,4,5-trisphosphate receptor) (Type 2 InsP3 receptor) (IP3 receptor isoform2) (InsP3R2) (Inositol 1,4,5-trisphosphate type V receptor).		2	2	NA
RB_MOUSE	P13405	Retinoblastoma-associated protein (PPI05)(RB).		2	2	NA
VP13A_MOUSE	Q5H8C4	Vacuolar protein sorting-associated protein 13A (Chorein) (Chorea-acanthocytosis protein homolog).		2	2	NA
YETS2_MOUSE	Q3TUF7	YEATS domain-containing protein 2.		2	2	NA
EZRI_RAT	P31977	Ezrin (p81) (Cytovillin) (Villin-2).		2	2	NA
CO9_RAT	Q62930	Complement component C9 precursor.		2	2	NA
CACP_RAT	Q704S8	Carnitine O-acetyltransferase (EC 2.3.1.7) (Carnitine acetylase) (CAT) (Carnitine acetyltransferase) (CrAT).		2	2	NA
ZSWM6_MOUSE	Q80TB7	Zinc finger SWIM domain-containing protein 6 (Fragment).		2	2	NA
TGFI1_MOUSE	Q3YBZ6	Transforming growth factor beta-1-induced transcript 1 protein (Hydrogen peroxide-inducible clone 5 protein) (Hic-5) (Androgen receptor-associated protein of 5 kDa) (TGF-beta-stimulated clone 5) (TSC-5).		2	2	NA
ADAT3_RAT	Q561R2	tRNA-specific adenosine deaminase 3 (tRNA-s specific adenosine-34 deaminase subunit ADAT3).		2	2	NA
HD_RAT	P51111	Huntingtin (Huntington disease protein homolog) (HD protein).		2	2	NA
MIA3_MOUSE	Q8BI84	Melanoma inhibitory activity protein 3 precursor (Protein TANGO).		2	2	NA
MYH4_RAT	Q29RW1	Myosin-4 (Myosin heavy chain 4) (Myosin heavy chain 2b) (MyHC-2b).		2	2	NA
MYH6_RAT	P02563	Myosin-6 (Myosin heavy chain 6) (Myosin heavy chain, cardiac muscle alpha isoform) (MyHC-alpha).		2	2	NA
DPOL1A_MOUSE	P33609	DNA polymerase alpha catalytic subunit (EC 2.7.7.7).		2	2	NA
CD302_RAT	Q5FVR3	CD302 antigen precursor (C-type lectin domain family 13 member A).		2	2	NA
MUC2_RAT	Q62635	Mucin-2 precursor (Intestinal mucin-2) (Fragment).		2	2	NA

Protein	Accession	Description	Scan Count	Irradiated	Control	Ratio
TGM1 RAT	P23606	Protein-glutamine gamma-glutamyltransferase K (EC 2.3.2.13)(Transglutaminase K) (TGase K) (TGK) (TG(K)) (Transglutaminase-1) (Epidermal TGase).	2	2	2	NA
NHERF RAT	Q9J119	Ezrin-radixin-moesin-binding phosphoprotein 50 (EBP50) (Na(+)/H(+) exchange regulatory cofactor NHERF-1) (Regulatory cofactor of Na(+)/H(+) exchanger) (Sodium-hydrogen exchanger regulatory factor 1)(Solute carrier family 9 isoform 3 regulatory factor 1).	2	2	2	NA
NOTC4 MOUSE	P31695	Neurogenic locus notch homolog protein 4 precursor (Notch 4)(Contains: Transforming protein Int-3; Notch 4 extracellular truncation; Notch 4 intracellular domain).	2	2	2	NA
WNK1 MOUSE	P83741	Serine/threonine-protein kinase WNK1 (EC 2.7.11.1) (Protein kinase with no lysine 1) (Protein kinase, lysine-deficient 1).	2	2	2	NA
ACHA RAT	P25108	Acetylcholine receptor subunit alpha precursor.	1	1	1	NA
RECO4 MOUSE	Q75NR7	ATP-dependent DNA helicase O4 (EC 3.6.1.-) (RecQ protein-like 4).	1	1	1	NA
MYH4 MOUSE	O5SX39	Mvosin-4 (Mvosin heavy chain 4) (Mvosin heavy chain 2b) (MvHC-2b).	1	1	1	NA
MERTK RAT	P57097	Proto-oncogene tyrosine-protein kinase MER precursor (EC 2.7.10.1) (C-mer) (Receptor tyrosine kinase MerTK).	1	1	1	NA
IF4G1 MOUSE	O6NZJ6	Eukaryotic translation initiation factor 4 gamma 1 (eIF-4-gamma 1)(eIF-4G1) (eIF-4G1).	1	1	1	NA
GNS MOUSE	Q8BFR4	N-acetylglucosamine-6-sulfatase precursor (EC 3.1.6.14) (G6S)(Glucosamine-6-sulfatase).	1	1	1	NA
DAG1 MOUSE	Q62165	Dystroglycan precursor (Dystrophin-associated glycoprotein 1)(Contains: Alpha-dystroglycan (Alpha-DG); Beta-dystroglycan (Beta-DG)).	1	1	1	NA
DGLA RAT	Q5YLM1	Sn1-specific diacylglycerol lipase alpha (EC 3.1.1.1.-) (DGL-alpha)(Neural stem cell-derived dendrite regulator).	1	1	1	NA
MARE3 RAT	Q5XIT1	Microtubule-associated protein RP/EB family member 3 (End-binding protein 3) (EB3)(EB1 protein family member 3) (EBF3) (RP3).	1	1	1	NA
PPID RAT	Q6DGG0	40 kDa peptidyl-prolyl cis-trans isomerase (EC 5.2.1.8) (PPIase)(Rotamase) (Cyclophilin-40) (CYP-40).	1	1	1	NA
ADCY5 RAT	Q04400	Adenylate cyclase type 5 (EC 4.6.1.1) (Adenylate cyclase type V) (ATPpyrophosphate-lyase 5) (Adenylate cyclase 5).	1	1	1	NA
BASP RAT	Q05175	Brain acid soluble protein 1 (BASP1 protein) (Neuronal axonal membrane protein NAP-22) (22 kDa neuronal tissue-enriched acidic protein).	1	1	1	NA
LAC2 RAT	P20767	Ig lambda-2 chain C region.	1	1	1	NA
CATC RAT	P80067	Dipeptidyl-peptidase 1 precursor (EC 3.4.14.1) (Dipeptidyl-peptidase) (DPP-1) (Cathepsin C) (Cathepsin J) (Dipeptidyl transferase) (Contains: Dipeptidyl-peptidase 1 exclusion domain chain (Dipeptidyl-peptidase 1 exclusion domain chain); Dipeptidyl-peptidase 1 heavy chain (Dipeptidyl-peptidase 1 heavy chain); Dipeptidyl-peptidase 1 light chain (Dipeptidyl-peptidase 1 light chain)).	1	1	1	NA
NOS2 MOUSE	P29477	Nitric oxide synthase, inducible (EC 1.14.13.39) (NOS type II)(Inducible NO synthase) (Inducible NOS) (Macrophage NOS) (MAC-NOS).	1	1	1	NA
UROM MOUSE	Q91X17	Uromodulin precursor (Tamm-Horsfall urinary glycoprotein) (THP).	1	1	1	NA
KNT1 RAT	P01048	T-kininogen 1 precursor (T-kininogen I) (Major acute phase protein)(Alpha-1-MAP) (Thioistatin) (Contains: T-kininogen 1 heavy chain (T-kininogen I heavy chain); T-kinin: T-kininogen 1 light chain (T-kininogen I light chain)).	21	20	20	120.00
CO4 RAT	P08649	Complement C4 precursor [Contains: Complement C4 beta chain; Complement C4 alpha chain; C4a anaphylatoxin; Complement C4 gamma chain].	16	15	15	115.00
KNT2 RAT	P08932	T-kininogen 2 precursor (T-kininogen II) (Major acute phase protein)(Alpha-1-MAP) (Thioistatin) [Contains: T-kininogen 2 heavy chain (T-kininogen II heavy chain); T-kinin: T-kininogen 2 light chain (T-kininogen I light chain)].	19	17	17	28.50
FINC RAT	P04937	Fibronectin precursor (FN).	355	307	307	486.40
AIAG RAT	P02764	Alpha-1-acid glycoprotein precursor (Orosomucoid) (OMD).	1612	1378	2345.89	2345.89
LIMC1 MOUSE	Q3UH68	LIM and calponin homology domains-containing protein 1.	18	15	15	35.00
CLUS RAT	P05371	Clusterin precursor (Sulfated glycoprotein 2) (SGP-2) (Dimeric acid glycoprotein) (DAG) (Testosterone repressed prostate message 2) (TRPM-2) [Contains: Clusterin beta chain; Clusterin alpha chain].	6	5	5	15.00
PSCL RAT	P02782	Prostatic steroid-binding protein C1 precursor (Prostatein peptide C1).	6	5	5	15.00
DNAS1 RAT	P21704	Deoxyribonuclease-1 precursor (EC 3.1.21.1) (Deoxyribonuclease I) (DNase I).	376	301	301	754.01
UROM RAT	P27590	Uromodulin precursor (Tamm-Horsfall urinary glycoprotein) (THP).	369	295	295	743.99
MEPIA RAT	Q64230	Meprin A subunit alpha precursor (EC 3.4.24.18) (Endopeptidase-24, 18 subunit alpha) (E-24, 18).	71	56	56	153.73
CAD13 MOUSE	Q9WTR5	Cadherin-13 precursor (Truncated-cadherin) (T-cadherin) (T-cad)(Heart-cadherin) (H-cadherin).	9	7	7	23.50
SODC RAT	P07632	Superoxide dismutase [Cu-Zn] (EC 1.15.1.1).	236	183	183	533.45
XLKD1 MOUSE	Q8BHC0	Lymphatic vessel endothelial hyaluronate acid receptor 1 precursor (LYVE-1) (Cell surface retention sequence-binding protein 1) (CRSBP-1) (Extracellular link domain-containing protein 1).	138	105	105	333.18
HEMO RAT	P20059	Hemopexin precursor.	713	539	539	1743.10
UP2 RAT	P81828	Urinary protein 2 precursor (RUP-2).	5599	4217	4217	13823.05
GELS RAT	Q68FP1	Gelsolin precursor (Actin-depolymerizing factor) (ADF) (Brevin).	186	140	140	463.04
CYTC RAT	P14841	Cystatin-C precursor (Cystatin-3).	141	106	106	353.03
CD48 RAT	P10252	CD48 antigen precursor (MRC OX-45 surface antigen) (BCM1 surface antigen) (BLAST-1).	4	3	3	13.00
CATB RAT	P00787	Cathepsin B precursor (EC 3.4.22.1) (Cathepsin B1) (RS G-2) [Contains: Cathepsin B light chain; Cathepsin B heavy chain].	50	37	37	132.85

Protein	Accession	Description	Scan Count	Irradiated	Control	Ratio
CD44 RAT	P26051	CD44 antigen precursor (Phagocytic glycoprotein 1) (PGP-1) (HUTCH-1)(Extracellular matrix receptor III) (ECMR-III) (GP90 lymphocytoheming/adhesion receptor) (Hermes antigen) (Hyaluronate receptor).	83	60		232.61
VTNC MOUSE	P29788	Vitronectin precursor (Serum-spreading factor) (S-protein).	36	26		102.60
UP3 RAT	P83121	Urinary protein 3 precursor (RUP-3).	6234	4445		17892.48
K2C6A RAT	Q4FZU2	Keratin, type II cytoskeletal 6A (Cytokeratin-6A) (CK 6A) (K6keratin).	58	41		172.41
K2C5 RAT	O6P6Q2	Keratin, type II cytoskeletal 5 (Cytokeratin-5) (CK-5) (Keratin-5)(K5).	51	36		152.40
PTGDS RAT	P22057	Prostaglandin-H 2 D-isomerase precursor (EC 5.3.99.2) (Lipocalin-type prostaglandin-D synthase) (Glutathione-independent PGD synthetase)(Prostaglandin-D2 synthase) (PGD2 synthase) (PGDS2) (PGDS).	34	24		102.40
UBIQ RAT	P62989	Ubiquitin.	37	26		112.36
GGT1 RAT	P07314	Gamma-glutamyltranspeptidase 1 precursor (EC 2.3.2.2) (Gamma-glutamyltransferase 1) (GGT 1) (CD224 antigen) [Contains: Gamma-glutamyltranspeptidase 1 heavy chain; Gamma-glutamyltranspeptidase 1 light chain].	37	25		122.08
UPI RAT	P81827	Urinary protein 1 precursor (RUP-1) (Liver regeneration-related protein LRRG05).	6817	4574		22432.04
KV2A7 MOUSE	P01631	Ig kappa chain V-II region 26-10.	99	66		332.00
PCLO MOUSE	Q9QYX7	Protein piccolo (Aczonin) (Multidomain presynaptic cytomatrix protein) (Brain-derived HLMN protein).	6	4		22.00
CADH2 RAT	Q9Z1Y3	Cadherin-2 precursor (Neural-cadherin) (N-cadherin) (CD325 antigen).	6	4		22.00
NCA11 MOUSE	P13595	Neural cell adhesion molecule 1, 180 kDa isoform precursor (N-CAM 180)(NCAM-180) (CD56 antigen).	3	2		12.00
IL4RA RAT	Q63257	Interleukin-4 receptor alpha chain precursor (IL-4R-alpha) (CD124 antigen).	94	57		371.54
DTX4 MOUSE	Q6PDK8	Protein deltex-4 (Deltex-4) (Deltex4).	10	6		41.50
SAP RAT	P10960	Sulfated glycoprotein 1 precursor (SGP-1)(Prosaposin).	10	6		41.50
MUP RAT	P02761	Major urinary protein precursor (MUP) (Alpha-2u-globulin) (Alpha (2)-euglobulin) (Allergen Rat n 1) (Rat n 1) [Contains: 15.5 kDa fatty acid-binding protein (15.5 kDa FABP)].	30355	17752		126031.41
EGF RAT	P07522	Pro-epidermal growth factor precursor (EGF) [Contains: Epidermal growth factor].	242	140		1021.37
KLK10 RAT	P36375	Glandular kallikrein-10 precursor (EC 3.4.21.35) (Tissue kallikrein) (T-kininogenase) (K10) (Proteinase B) (Endopeptidase K) [Contains: T-kininogenase light chain; T-kininogenase heavy chain].	14	8		61.33
TRY1 RAT	P00762	Anionic trypsin-1 precursor (EC 3.4.21.4) (Anionic trypsin 1) (Pretyrpsinogen 1) (Serrine protease 1).	3600	2004		15961.26
		Nerve growth factor gamma chain precursor (EC 3.4.21.35) (Gamma-NGF) (Tissue kallikrein) (True tissue kallikrein) (True kallikrein) (PSkallikrein) (RGK-1) (rK-1) (Pancreatic kallikrein) [Contains: Nerve growth factor gamma chain 1; Nerve growth factor gamma chain 2].				
KLK1 RAT	P00758	Parvalbumin alpha.	133	74		591.25
PRVA RAT	P02625	Major urinary protein (MUP) (Alpha-2u-globulin) (Alpha (2)-euglobulin) (Fragments).	29	16		131.23
MUP RATRT	P84855	Major urinary protein precursor (CBG) (Transcortin) (SerpinaA6).	188	103		851.21
CBG RAT	P31211	Corticosteroid-binding globulin precursor (CBG) (Transcortin) (SerpinaA6).	50	27		231.17
PS2 RAT	P02781	Prostatic steroid-binding protein C2 precursor (Prostatein peptide C2).	45	24		211.14
CCGS RAT	Q8VHW8	Voltage-dependent calcium channel gamma-5 subunit (Neuronal voltage-gated calcium channel gamma-5 subunit).	61	32		291.10
PI16 MOUSE	Q9ET66	Peptidase inhibitor 16 precursor (Cysteine-rich protease inhibitor).	35	18		171.06
AMYS MOUSE	P00687	Alpha-amylase 1 precursor (EC 3.2.1.1) (1,4-alpha-D-glucanoglucanohydrolase) (Salivary and hepatic alpha-amylase).	45	23		221.05
HA12 RAT	P16391	RT1 class I histocompatibility antigen, AA alpha chain precursor.	2	1		11.00
PEBP1 RAT	P31044	Phosphatidylethanolamine-binding protein 1 (PEBP-1) (HCNPPp) (23 kDa morphine-binding protein) (P23K) [Contains: Hippocampal cholinergic neurostimulating peptide (HCNP)].				
SP21 RAT	P09005	Serine protease inhibitor 2.1 (SPI-2.1) (Fragment).	2	1		11.00
ELOV1 MOUSE	Q9JLJ5	Elongation of very long chain fatty acids protein 1.	332	164		1680.98
SPA3L RAT	P05544	Serine protease inhibitor A3L precursor (Serpina A3L) (Contrapsin-like protease inhibitor 3) (CPL-23) (Serine protease inhibitor 1) (SPI-1).	21	9		120.75
PARP1_MOUSE	P11103	Poly (ADP-ribose) polymerase 1 (EC 2.4.2.30) (PARP-1) (ADPRT) (NAD(+)ADP-ribose transferase 1) (Poly[ADP-ribose] synthetase 1) (msPARP).	2672	1115		15570.72
		Epithelial-cadherin precursor (E-cadherin) (Uvomortilin) (Cadherin-1) (CD324 antigen) [Contains: E-Cad/CTF1; E-Cad/CTF2; E-Cad/CTF3].	42	17		250.68
CADH1 RAT	Q9ROT4	Gamma-glutamyl hydrolase precursor (EC 3.4.19.9) (Gamma a-Glu-Xcarboxypeptidase) (Conjugase) (GH).	158	62		960.65
GGH RAT	Q62867	Basement membrane-specific heparan sulfate proteoglycan core protein precursor (HSPG) (Perlecan) (PLC).	44	17		270.63
PGEM MOUSE	Q05793	Alpha-2-HS-glycoprotein precursor (Fetuin-A) (Glycoprotein PP63) (59kDa bone sialic acid-containing protein) (BSP).	13	5		80.63
FETUA RAT	P24090	Mucin and cadherin-like protein precursor (Mu-protocadherin) (GP100).	32	12		200.60
MUCDL RAT	Q9JIK1	Ig kappa chain C region, A allele.	8	3		50.60
KACA RAT	P01836	Major urinary protein 1 precursor (MUP 1).	1322	495		8270.60
MUPI MOUSE	P11588	Ig kappa chain C region, B allele.	131	49		820.60
KACB RAT	P01835	Beta-2-microglobulin precursor.	1321	494		8270.60
B2MG RAT	P07151	Beta-2-microglobulin precursor.	141	50		910.55
BGLR RAT	P06760	Beta-glucuronidase precursor (EC 3.2.1.31).	17	6		110.55

Protein	Accession	Description	Scan Count	Irradiated	Control	Ratio
KLH24_RAT	Q56A24	Kelch-like protein 24 (Protein DRE1).	32	11	21	0.52
AMYP_MOUSE	P00688	Pancreatic alpha-amylase precursor (EC 3.2.1.1) (PA) (1,4-alpha-D-glucan glucanohydrolase).	12	4	8	0.50
CFAD_RAT	P32038	Complement factor D precursor (EC 3.4.21.46) (C3 convertase activator)(Properdin factor D) (Adipsin) (Endogenous vascular elastase).	9	3	6	0.50
P1GR_RAT	P15083	Polymeric-immunoglobulin receptor precursor (Poly-Ig receptor) (PIGR)[Contains: Secretory component].	3	1	2	0.50
SPA3K_RAT	P05545	Serine protease inhibitor A3K precursor (SerpA A3K) (Contrapsin-like protease inhibitor 1) (CPI-21) (Kallikrein-binding protein) (KBP) (Growth hormone-regulated proteinase inhibitor) (Serpine proteaseinhibitor 2) (SPI-2) (GHR-P63) (SPI-2 .3) (Thyroid hormone-regulatedprotein).	3059	995	2064	0.48
TRY2_MOUSE	P07146	Anionic trypsin-2 precursor (EC 3.4.21.4) (Anionic trypsin II)(Preturpsinogen II) (Serine protease 2).	42	13	29	0.45
GRPE_MOUSE	Q6GYPT	GTPase-activating RapGAP domain-like 1 (GAP-related-interactingpartner to E12) (GRPE) (Tuberin-like protein 1).	52	16	36	0.44
ATOX1_RAT	O9WUC4	Copper transport protein ATOX1 (Metal transport protein ATX1) (ATX1 homolog protein Rah1).	14	4	10	0.40
UNC5A_MOUSE	Q8K1S4	Netrin receptor UNC5A precursor (Unc-5 homolog A) (Unc-5 homolog 1).	7	2	5	0.40
AMBP_RAT	Q64240	AMBP protein precursor [Contains: Alpha-1-microglobulin; Inter-alpha-trypsin inhibitor light chain (ITI-LC) (Bikumin) (HI-30); Trypsin].	865	243	622	0.39
ALBU_MOUSE	P07724	Serum albumin precursor.	441	121	320	0.38
ANGT_RAT	P01015	Angiotensinogen precursor (SerpA A8) [Contains: Angiotensin-1(Angiotensin D) (Ang D); Angiotensin-2 (Angiotensin II) (Ang II);Angiotensin-3 (Angiotensin III) (Ang III) (Des-Asp1[1]-angiotensinII)].	15	4	11	0.36
SGP50_RAT	Q04807	Sulfated 50 kDa glycoprotein precursor (SGP50) (Endothelial ligand FORL-selectin) (Glycosylation-dependent cell adhesion molecule 1) (GlyCAM-1).	296	70	226	0.31
JAM1_RAT	Q9JHY1	Functional adhesion molecule A precursor (JAM-A) (Junctional adhesionmolecule 1) (JAM-1) (CD321 antigen).	9	2	7	0.29
MUG1_RAT	Q03626	Murineoglobulin-1 precursor (Alpha-1 inhibitor 3 variant D) (Alpha-Xprotein).	9	2	7	0.29
MUP3_MOUSE	P04939	Major urinary protein 3 precursor (MUP 3) (Non-group 1/group 2 MUP15).	5	1	4	0.25
AIAT_RAT	P17475	Alpha-1-antitrypsin precursor (Alpha-1-antitrypsin) (Alpha-1-proteinase inhibitor).	113	21	92	0.23
OSMR_MOUSE	Q70458	Oncostatin-M specific receptor subunit beta precursor.	11	2	9	0.22
ALBU_MOUSE	P02770	Serum albumin precursor.	1854	302	1552	0.19
LRC33_MOUSE	Q8BMT4	Leucine-rich repeat-containing protein 33 precursor.	19	3	16	0.19
TRFE_RAT	P12346	Serotransferrin precursor (Transferrin) (Siderophilin) (Beta-1-metal-binding globulin) (Liver regeneration-related protein LRRG03).	13	2	11	0.18
SNX8_MOUSE	Q8CFD4	Sorting nexin-8.	14	2	12	0.17
BPAL_MOUSE	Q91ZU6	Bullous pemphigoid antigen 1, isoforms 1/2/3/4 (BPA) (Hemidesmosomalplaque protein) (Dystonia musculorum protein) (Dystonin).	15	2	13	0.15
ASHIL_MOUSE	Q99MY8	Probable histone-lysine N-methyltransferase ASH1L (EC 2.1.1.43) (ASH1-like protein) (Absent small and homeotic disks protein 1 homolog).	77	10	67	0.15
AT10A_MOUSE	Q54827	Probable phospholipid-transporting ATPase VA (EC 3.6.3.1) (P-locusfat-associated ATPase).	74		74	NA
BRCA1_MOUSE	P48754	Breast cancer type 1 susceptibility protein homolog.	68		68	NA
TLN2_MOUSE	Q71LX4	Talin-2.	49		49	NA
TACC3_MOUSE	Q9J111	Transforming acidic coiled-coil-containing protein 3 (ARNT-interactingprotein).	40		40	NA
DIDO1_MOUSE	Q80V34	Death-inducer obliterator 1 (DIO-1) (Death-associated transcriptionfactor 1) (DATF-1).	23		23	NA
B2MG_CAVPO	P01886	Beta-2-microglobulin.	18		18	NA
LRPIB_MOUSE	Q9J118	Low-density lipoprotein receptor-related protein 1B precursor (Low-density lipoprotein receptor-related protein-deleted in tumor) (LRP-DIT).	17		17	NA
ECM29_MOUSE	Q6PDI5	Proteasome-associated protein ECM29 homolog (Ecm29).	15		15	NA
MPRI_MOUSE	Q07113	Cation-independent mannose-6-phosphate receptor precursor (CI Man-6-Preceptor) (CI-MPR) (M6PR) (Insulin-like growth factor 2 receptor)(Insulin-like growth factor II receptor) (IGF-II receptor) (M6P/IGF2receptor) (M6P/IGF2R) (300 kDa mannose 6-phosphate receptor) (MPR 300)(MPR300).	12		12	NA
FLNC_MOUSE	Q8VHX6	Filamin-C (Gamma-filamin) (Filamin-2) (FLN-C) (Actin-binding-likeprotein) (ABP-L) (ABP-280-like protein).	11		11	NA
IQEC1_MOUSE	Q8R0S2	IQ motif and Sec7 domain-containing protein 1.	11		11	NA
SG2A2_RAT	P02780	Secretoglobulin family 2A member 2 precursor (Prostatic steroid-bindingprotein C3) (C3.1) (Prostatein peptide C3).	8		8	NA
AIAT_CALCN	Q54763	Alpha-1-antitrypsin precursor (Alpha-1-antitrypsin) (Alpha-1-proteinase inhibitor).	8		8	NA
JHD2C_MOUSE	Q69ZK6	Probable JmiC domain-containing histone demethylation protein 2C(EC 1.14.11.-) (Jumonji domain-containing protein 1C).	7		7	NA
AFAM_RAT	P36953	Afamin precursor (Alpha-albumin) (Alpha-Alb).	7		7	NA
GOGA4_MOUSE	Q91VW5	Golgin subfamily A member 4 (Golgin-1).	6		6	NA
NU155_RAT	P37199	Nuclear pore complex protein Nup155 (Nucleoporin Nup155) (155 kDa nucleoporin) (P140).	6		6	NA
VITRN_MOUSE	Q8VHI5	Vitrin precursor.	6		6	NA
APOB_RAT	Q7TMA5	Apolipoprotein B-100 precursor (Apo B-100) [Contains: ApolipoproteinB-48 (Apo B-48)].	6		6	NA
NAGS_MOUSE	Q8R4H7	N-acetylglutamate synthase, mitochondrial precursor (EC 2.3.1.1) (Amino-acid acetyltransferase) [Contains: N-acetylglutamate synthase; long form; N-acetylglutamate synthase short form; N-acetylglutamate synthase conserved domain form].	6		6	NA

Protein	Accession	Description	Scan Count	Irradiated	Control	Ratio
RIN3_MOUSE	Q8K2R5	Zinc finger protein 668.	5			5NA
RIN3_MOUSE	P59729	Ras and Rab interactor 3 (Ras interaction/interference protein 3).	5			5NA
MAGDI_MOUSE	Q9QYH6	Melanoma-associated antigen DI (MAGE-D1 antigen) (Neurotrophinreceptor-interacting MAGE homolog) (Dixin-1).	5			5NA
MUC4_RAT	Q63661	Mucin-4 precursor (Pancreatic adenocarcinoma mucin) (Testis mucin) (Asciates sialoglycoprotein) (ASGP) (Sialomucin complex) (Pre-sialomucin complex) (pSMC) [Contains: Mucin-4 alpha chain (Asciatesialoglycoprotein 1) (ASGP-1); Mucin-4 beta chain (Asciatesialoglycoprotein 2) (ASGP-2)].				
TGON3_RAT	P19814	Trans-Golgi network integral membrane protein TGN38 precursor.	5			5NA
LRIG2_MOUSE	Q52KR2	Leucine-rich repeats and immunoglobulin-like domains protein 2precursor.	5			5NA
AMYP_RAT	P00689	Pancreatic alpha-amylase precursor (EC 3.2.1.1) (PA) (1,4-alpha-D-glucan glucanohydrolase).	4			4NA
ZN294_MOUSE	Q6A009	Zinc finger protein 294 (Zfp-294).	4			4NA
RISC_RAT	Q920A6	Retinoid-inducible serine carboxypeptidase precursor (EC 3.4.16.-)(Serine carboxypeptidase 1).	4			4NA
CFI36_RAT	Q6MG12	Uncharacterized protein C6orf136 homolog.	4			4NA
SPA3M_RAT	Q6S556	Serine protease inhibitor A3M precursor (Serpin A3M) (Serine proteaseinhibitor 2.4) (SPI-2.4) (Fragment).	4			4NA
AIM_RAT	Q63041	Alpha-1-macroglobulin precursor (Alpha-1-M) [Contains: Alpha-1-macroglobulin 165 kDa subunit; Alpha-1-macroglobulin 45 kDa subunit].	4			4NA
NID1_RAT	P08460	Nidogen-1 (Entactin) (Fragment).	3			3NA
GAK_RAT	P97874	Cyclin G-associated kinase (EC 2.7.11.1).	3			3NA
CP4F1_RAT	P33274	Cytochrome P450 4F1 (EC 1.14.14.1) (CYP1VF1) (P450-A3).	3			3NA
KV5A3_MOUSE	P01635	Ig kappa chain V-V region K2 precursor (Fragment).	3			3NA
FZD8_RAT	Q498S8	Frizzled-8 precursor (Fz-8).	3			3NA
POLK_MOUSE	Q9OUG2	DNA polymerase kappa (EC 2.7.7.7) (DINB protein) (DINP).	3			3NA
KLH20_MOUSE	Q8VCK3	Kelch-like protein 20 (Kelch-like ECT2-interacting protein).	3			3NA
FUBP2_RAT	Q99PF5	Far upstream element-binding protein 2 (FUSE-binding protein 2) (KHtype-splicing regulatory protein) (KSRP) (MAP2 RNA trans-actingprotein 1) (MAPTA1).	3			3NA
CP4F3_RAT	Q3MID2	Cytochrome P450 4F3 (EC 1.14.13.30) (CYP1VF3) (Leukotriene-B(4) omega-hydroxylase) (Leukotriene-B(4) 20-monooxygenase)	3			3NA
C1Q74_MOUSE	Q8R066	Complement C1q tumor necrosis factor-related protein 4 precursor.	2			2NA
A4_RAT	P08592	Anyloid beta A4 protein precursor (APP) (ABPP) (Alzheimer diseaseamyloid protein homolog) (Amyloidogenic glycoprotein) (AG) [Contains: Soluble APP-alpha (S-APP-alpha); Soluble APP-beta (S-APP-beta); C99;Beta-amyloid protein 42 (Beta-APP42); Beta-amyloid protein 40 (Beta-APP40); C83; P3(42); P3(40); Gamma-secretase C-terminal fragment 59); Gamma-CTF(57) (Gamma-secretase C-terminal fragment 57);Gamma-CTF(50) (Gamma-secretase C-terminal fragment 50); C51].	2			2NA
MY18A_MOUSE	Q811D7	Myosin-XVIIIa (Mvosin containing a PDZ domain) (Molecule associatedwith JAK3 N-terminus) (MAJN).	2			2NA
EMAL3_MOUSE	Q8VC03	Echinoderm microtubule-associated protein-like 3 (EMAP-3).	2			2NA
RBP2_MOUSE	Q9ERU9	E3 SUMO-protein ligase RbBP2 (Ran-binding protein 2).	2			2NA
CC113_MOUSE	Q8C5T8	Coiled-coil domain-containing protein 113.	2			2NA
3MG_RAT	P23571	DNA-3-methyladenine glycosylase (EC 3.2.2.21) (3-methyladenine DNAglycosidase) (ADPG) (3-alkyladenine DNA glycosylase) (N-methylpurine-DNA glycosylase) (Fragment).	2			2NA
DSG2_MOUSE	Q55111	Desmoglein-2 precursor.	1			1NA
NRP2_RAT	Q35276	Neuropilin-2 precursor (Vascular endothelial cell growth factor 165receptor2).	1			1NA
APOA4_RAT	P02651	Apolipoprotein A-IV precursor (Apo-AIV) (ApoA-IV).	1			1NA
CS010_MOUSE	Q9CPT4	Uncharacterized protein C19orf10 homolog precursor (Stromal cell-derived growth factor SF20) (Interleukin-25) (IL-25).	1			1NA
EFNB2_MOUSE	P52800	Ephrin-B2 precursor (EPH-related receptor tyrosine kinase ligand 5)(L-ERK-5) (HTK ligand) (HTK-L) (ELF-2).	1			1NA
CALB1_RAT	P07171	Calbindin (Vitamin D-dependent calcium-binding protein, avian-type)(Calbindin D28) (D-28K) (Spot 35 protein).	1			1NA
CAD11_MOUSE	P55288	Cadherin-11 precursor (Osteoblast-cadherin) (OB-cadherin) (OSF-4).	1			1NA