Developing omics-based approaches for short- and long-term space radiation risk assessment

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A. **Overview**. The ability to model estimates of space-radiation-associated cancer risks to astronauts on long-term missions in space is complex. The completion of the human genome project about 10 years ago was a defining and important step towards the beginning of our understanding how the human organism works. Using a variety of "omics" approaches to compare cells, tissues and animal models and putting them together into systems biology concepts is beginning to emerge. The term "omics" refers to the application of global profiling and analysis approaches to study a general area of biology, such as genomics, transcriptomics, proteomics, and metabolomics. In addition to these major categories, there are an increasing number of "omics" categories and subcategories such as lipidomics (non-polar metabolites), epigenomics, glycoproteomics and many others. Integrating analyses from these various "omics" analyses into a systems biology understanding of biomedical and fundamental biological processes is an important overall goal (**Figure 1**)[1], and has led to the term "integromics" which allows biomedical scientists with informatics expertise to be able to integrate and use the information fluently for both hypothesis generation, delineation of biomarkers for injury and

[1] All figures and legends are shown after the References section.

disease, and for modeling risks (as discussed by Weinstein [1]). Systems biology is an inter-disciplinary field of study that focuses on complex interactions within biological systems, using a more holistic perspective rather than the more typical reductionism approach to biomedical research. The overarching goal of systems biology is to model and discover properties of cells, tissues and organisms functioning as a system. In this general overview of the "omics" field we will present how space radiation researchers can address the complexities of modeling cancer risk assessment primarily using cells and mouse models of human disease.

The scope or alphabet of the "omics" space is summarized in **Figure 2**, and highlights the complexity at each level. While there are about twenty thousand coding genes in the human genome, alternate splicing gives rise to a substantially greater number of mRNA transcripts plus many other transcripts that do not encode proteins (non coding RNAs). An estimate of the number of different proteins is daunting since there are a myriad of processing events. If one includes the potential number of various post-translational modifications, such as phosphorylation and acetylation, then the number of distinct different proteins is nearly unlimited. In the case of small molecules, typical metabolic maps often illustrate a few thousand but the actual number of different endogenous metabolites or small molecules is certainly much greater. If metabolites or measuring metabolic flux from the environment and the microbiome are included, then the number easily approaches a hundred thousand.

A major challenge for human space missions is radiation exposure. Long duration space missions will expose astronauts to radiation that is different in dose and quality compared to terrestrial radiation. Radiation on earth is mostly low linear energy transfer (low-LET) radiation such as γ-rays or x-rays, which are sparsely ionizing. On the contrary, astronauts traveling beyond low-earth orbit (LEO) are expected to encounter a mixed radiation field, which includes

high-energy proton and heavy ion radiation at low doses and dose rates. Of major concern to NASA mission planners is exposure to high-Z and high-energy (HZE) particle radiation also known as heavy ion radiation such as ^{56}Fe, ^{28}Si, ^{16}O, and ^{12}C, which are considered high-LET and densely ionizing. However, much uncertainty exists about adverse short- as well as long-term consequences of space radiation especially HZE radiation exposure on human health due to lack of reliable risk prediction models. One general approach for risk assessment is to determine the relative biologic effectiveness (RBE) of various parameters for space radiation compared to terrestrial γ radiation exposures. Since there is sufficient statistical sampling for the latter, risk estimates can then be "extrapolated" to space radiation using a RBE scaling factor; needless to say, the reliability of the models for RBE determination is the key. Development of a reliable space radiation risk prediction model is hampered due to paucity of *in vivo* mechanistic data on tissue specific consequences of space radiation in human or in animal models. Considering limitations in acquiring adequate human data, an integrated omics-based approach using appropriate mouse models could aid in the development of accurate tissue specific long-term cancer risk estimates for astronauts who will encounter persistent space radiation exposures during long duration deep space exploratory missions. An omics-based approach will also allow identification of health risk markers associated with space radiation exposure at the genomic, transcriptomic, proteomic, and metabolomic level.

B. **Genomics and epigenomics**. Genomics involves modern high-throughput and high-density DNA sequencing to understand global structural and functional alterations at the genome level. We utilize Illumina DNA sequencing technology, which relies on the attachment of randomly fragmented genomic DNA to a planar, optically transparent surface. Attached DNA fragments

are extended and bridge amplified to create an ultra-high density sequencing flow cell with >50 million clusters, each containing ~1,000 copies of the same template. These templates are sequenced using a robust four-color DNA sequencing-by-synthesis technology that employs reversible terminators with removable fluorescent dyes. This novel approach ensures high accuracy and true base-by-base sequencing, eliminating sequence-context specific errors and importantly determination of the landscape of genes mutated after low and high-LET irradiation. While we expect many random mutations from irradiation, our hypothesis is that specific gene mutations will be overrepresented in cells that show cancer progression biological endpoints. Thus, by sequencing normal colonic epithelial and colonic stem cells we may identify non-random mutations in tumors that emerge. These candidates can then be compared to spontaneous tumors and tumors produced after low versus high-LET irradiation in mouse models of gastrointestinal (GI) cancer. We are rapidly approaching the $1000 next generation sequencing genome making studies much more approachable and affordable.

Another commonly used technique is comparative genomic hybridization (CGH) microarray, which is created by imprinting of small DNA probes (25–85 base pairs) or genomic clones for specified positions in the genome. Extracted DNA from samples including irradiated sample (e.g., blood and various tissues) are first labeled with a fluorescent dye, while DNA from a normal control sample is labeled with a different dye, then mixed together and applied to a microarray for hybridization. The fluorescence intensity ratio from the irradiated and control group is determined at specified positions along the genome and provides a relative estimates of the copy number of a specific gene in the irradiated genome as compared to the control. Chromosomal amplifications, deletions and aberrations are considered important in radiation-induced carcinogenesis. However, very limited numbers of radiobiological studies have been

conducted using DNA sequencing and/or CGH array based approaches. A few studies focusing on radiation-induced carcinogenesis of thyroid, mammary gland and lymph tissues are available following low-LET radiation exposure. In immortalized human thyroid epithelial cells exposed to γ radiation a predominant pattern of sub-telomeric deletions in chromosome 10 has been observed [2]. In addition, frequent loss of the IgH-region on chromosome 12, 4 and 14 were significantly associated with the irradiated group along with copy number imbalances of other chromosomal regions has been implicated in lymphomagenesis after continuous low dose rate irradiation [3]. Distinct DNA copy number aberration patterns were seen in 2 Gy γ-radiation-induced mammary carcinoma compared to spontaneous mammary tumors [4]. Although genomics-based approaches are important and there are low-LET radiation-induced mutagenesis and chromosomal aberrations studies, we have yet to have sufficient *in vivo* data on genomic alterations following space radiation exposure.

In contrast to DNA sequencing to identify nucleotide base mutations in the genome, epigenetics (non-DNA sequence changes) provide information on chemical modifications to DNA of an organism which are heritable and do not involve a change in nucleotide sequence. However, epigenetic changes can alter the function of the genome. The term epigenome means "above the genome or over genetics" and refers to stable, long-term alterations in the transcriptional potential of a cell. Mechanisms that can produce such changes include DNA methylation and histone modifications. With genome-wide methylation arrays now available, we are beginning to understand complex traits such as susceptibility to disease and perhaps such studies may offer insights into the role of irradiation, gene expression changes and susceptibility to cancer in the future. Today there are many approaches to understanding the epigenome. These include genome-wide DNA methylation analysis, targeted sequencing for DNA methylation

analysis, ChIP-seq for chromatin immunoprecipitation assays followed by next generation sequencing to study histone modifications and protein-DNA interactions on a genome-wide scale. The key to understanding the importance of DNA sequencing and the epigenome is bioinformatic solutions for the analysis of raw data.

Recently available molecular analysis of space radiation response has demonstrated an important role of epigenetic changes mainly promoter DNA methylation, which may act as long-term determinants of gene expression and can drive persistent changes in the mechanisms involved in radiation carcinogenesis. In a recent study in mice at 1, 7, 30 and 120 days following ^{56}Fe ion exposure Lima and coworkers showed that the long-term differences in methylation profiles of carcinogenesis-related genes responses were tissue specific, and dose and time dependent [5]. In another study, ionizing radiation-induced microRNA (miRNA) expression involved in regulation of chromatin remodeling and DNA methylation could be interpreted to suggests that low-LET radiation induced higher number of miRNAs involved in epigenetic regulation compared to ^{56}Fe ion exposure [6]. Radiation epigenetics, especially space radiation epigenetics, is still an emerging discipline and further understanding of epigenetic changes and its regulatory factors after space radiation exposure is warranted to understand risks and design preventive strategies.

C. **Transcriptomics**. Radiation induces many types of damage to biomolecules particularly DNA, and the carcinogenic potential of radiation can be attributed to alterations in the expression of genes involved in proliferation that provide survival advantage to cells, as well as expression of mutated genes that can have many downstream effects. Changes in gene expression in response to radiation exposure have been reported to vary depending on the dose and radiation

quality; and there are significant differences in acute stress-types and delayed responses to radiation exposure [7]. Delayed effects of IR on gene expression are not only associated with cancer initiation and promotion in normal cells but are also known to play a critical role in the development of resistance to therapy in cancer cells. Considering the carcinogenic potential of radiation exposure to target tissues such as the lung and gastrointestinal tract, it is important to understand how radiation modulates long-term gene expression changes and how these changes relate to oncogenic signaling pathways known to be involved in cancer initiation/progression as well as resistance to therapy. We have developed approaches to assess a global view of the temporal changes in gene expression associated with space radiation exposure. This may allow us to identify novel target genes and regulated pathways, and should support not only data for modeling risk estimation but also clues for development of preventive strategies.

Representative results of transcriptomics analyses are shown in Figures 3 and 4. In Figure 3A results have been summarized in what is known as a heat map showing genes that either increase (red) or decrease (green) in murine intestinal tissue 2 months after either high-LET ^{56}Fe ion radiation or low-LET γ irradiation. Both types of radiation trigger long-term changes in gene expression relative to sham-irradiated controls with substantial overlap between the two types of radiation; e.g., the upper third of the heat map shows numerous genes that are decreased for both radiation types. However, ^{56}Fe and γ radiation responses differ substantially as well; e.g. in the lower portion of the heatmap, there are stronger responses after ^{56}Fe radiation relative to γ rays. Although, our current omics-based approaches to space radiation risk estimates involve lower radiation doses (<0.5 Gy), these initial experiments were performed in wild type mice at high radiation doses (6 Gy ^{56}Fe and 7 Gy γ radiation) with a limited number of mice to allow pronounced observable responses associated with heavy ion (^{56}Fe) space radiation and prepare

the platform for subsequent space radiation-induced gastrointestinal tumor analysis. Once radiation responsive genes have been identified, pathway analysis can be carried out to identify potential pathways regulating these responses. Signaling pathway alterations associated with the microarray data were performed using Ingenuity Pathway Analysis (IPA, Redwood City, CA), which uses a built-in library known as the Ingenuity Pathways Knowledge Base (IPKB) to identify biologically relevant signaling networks that are significantly perturbed in the uploaded gene expression data. While detailed microarray data presentation and discussion is beyond the scope of this review, we have shown two examples, one from γ and the other from ^{56}Fe samples, in Figure 3B and C to highlight the importance of transcriptomics in space radiation risk estimation. For γ radiation (Figure 3B), the top signaling network identified by IPA shows fibroblast growth factor 1 (FGF1) as the nodal molecule and FGF1 signaling has been demonstrated to be involved in mitogenic and angiogenic activity in different cell types [8]. Importantly, FGF1 via its receptor, FGFR1, activates a host of downstream signaling cascades including JNK/STAT, and MAPK/ERK leading to cell proliferation [8], which could play a role in γ radiation-induced carcinogenesis. For ^{56}Fe radiation, the top signaling network identified by IPA is shown in Figure 3C where genes related to NFκB signaling show increased expression in mouse intestinal tissue two months after radiation exposure. Although we did not observe upregulation of NFkB itself per se, we did observe upregulation of a number of NFkB pathway-associated signaling molecules such as STAT5, CXCL16, NDRG1, ICAM1, and LGR4, which are known to play critical roles in carcinogenesis. An additional example of radiation-induced transcriptomic upregulation in mouse mammary gland tissue 2 months after radiation exposure is shown in Figure 4 where again NFκB is the nodal molecule of the signaling network identified by IPA. It is important to note that NFκB is a well-known transcription factor involved in

regulation of genes involved in cell proliferation and survival responses [9, 10] and activation of NFκB is also known to accelerate carcinogenesis in response to genotoxic stressors such as radiation [9, 10]. NFkB is activated by an array of external stimuli including infection, inflammation, radiation, and oxidative stress resulting in upregulation of a number of downstream stress response genes such as iNOS, COX2, TNFα, and intracellular adhesion molecule (ICAM) [10]. Low-LET radiation-induced transcriptomic changes in different tissue and cell systems have been widely studied. However, only a handful of studies dealing with differential changes in mRNA and microRNA (miRNA) expression after low-LET and high-LET radiation are available to date. In a study by Kurpinski et al (2009) transcriptomics based pathway analyses revealed that DNA/RNA metabolism, DNA replication, DNA strand elongation and cell cycle regulatory transcripts were more highly perturbed after ^{56}Fe ions exposure than x-ray [11]. In addition to mRNA expression, miRNA signatures are also equally important to understand a particular biological phenomenon. An analysis of miRNA expression profiles from mouse peripheral blood after γ-rays, proton and ^{56}Fe-ions exposure revealed radiation type and dose specific expression signatures [12]. Additional studies at varying doses of different space radiation beams will be required to establish correlative changes in mRNA and/or miRNA expression in relation to low-LET radiation and characterize molecular signatures specific to space radiation.

D. Proteomics. The entire protein pool synthesized or modified by an organism is called the "proteome" and the large-scale study of functional and/or structural aspects of the entire protein pool is defined as "proteomics". Proteomics analysis can elucidate the organization and dynamics of the cellular signaling regulatory networks by which an organism or a cell responds

in the presence of stressors. Using proteomics, stress-induced alterations in proteins can be studied at expression, functional, and structural levels [13]. "Expression proteomics" is the quantitative and qualitative analysis of many expressed proteins under two or more different conditions. "Functional Proteomics" is used to identify protein molecules implicated in an individual metabolic activity and their contribution to the whole metabolic network. Functional proteomics provides datasets of signaling proteins, and pathway maps of their signaling interactions that helps to understand cellular signaling in relation to a specific biological condition. "Structural proteomics" is used to identify and characterize physical protein interactions present in a complex state such as ribosomes, membranes, and chromatin [14].

Proteomic analysis often involves mass spectrometry (**Figure 5**) and array (**Figure 6**) based techniques [15, 16]. Earlier approaches in proteomics were based on high resolution, two-dimensional gel electrophoresis, followed by selection and identification of differentially expressed proteins by mass spectrometry [15, 16], but increasingly the use of direct liquid chromatography-mass spectrometry methodologies are being employed. For example the approach in Figure 5 employs stable isotope tags to differentially label proteins from two different treatments or conditions. Here, a complex mixture of proteins is labeled and then digested to generate labeled peptides. The labeled peptide mixtures are then combined from two different treatment conditions, and separated by multidimensional liquid chromatography and analyzed by tandem mass spectrometry [17]. In addition to mass-spectrometry based approaches, protein arrays are alternatively used for large scale profiling of proteins [16]. Protein capture array (PCA) requires a library of antibodies arrayed on the chip surface and the array is probed with cell lysates or serum, and analysis of the resulting binding reactions using various detection systems provides information about expression levels of particular proteins in the sample as well

as measurements of binding affinities and specificities [18]. In addition, targeted protein arrays (TPA) are constructed by immobilizing known proteins on chip surfaces and are used to study protein-interactions (protein-protein, protein-nucleic acid, protein-phospholipid, and protein-small molecule interactions), to assay enzymatic activity, and to detect and validate antibodies. These protein chips are also used to study biochemical activities of the entire proteome in a single experiment. Moreover, reverse phase protein arrays (RPPA) can be used for complex samples, such as tissue lysates. A protein sample is arrayed onto slides and probed with known antibodies generated against the target protein. Signal detection can be done with chemi-luminescence, fluorescence, or colorimetric assays. Known proteins are printed on the slides that serve as reference for protein quantification in the unknown sample. RPPA can also be used for the determination of post-translationally modified proteins such as phosphorylation processes [19].

By comparing proteins expressed following radiation exposure with different radiation settings (i.e. radiation dose, exposure time and radiation in reference to their respective controls), it is possible to identify radiation-induced changes in biochemical signaling pathways. Using proteomics, it also possible to identify radiation exposure associated protein biomarkers and also to make deductions about regulatory networks by identifying proteins that undergo coordinated changes of expression.

Knowledge of the acute and delayed effects of low doses of radiation (e.g. bystander effect) is not yet fully established at the molecular level. Proteomics analysis and its correlation with other "omics" techniques, is believed to be a good way forward for identification of target signaling molecules, biomarkers as well as the physiological and health significance related to acute and chronic radiation exposures. Proteins and related signaling networks at the systems

level using serum, urine and tissue extracts have been widely studied in response to low-LET radiation. However, a very limited number of publications are available comparing low-LET effects with high-LET radiation. A few recent studies have been conducted using carbon-ion radiation, which has relevance in space radiation related risk estimates as well as in cancer radiotherapy. Proteomics analysis 14 days after whole body carbon-ion exposure in mice testes showed differentially expression proteins involved in energy supply, the endoplasmic reticulum, cell proliferation, cell cycle, antioxidant capacity and mitochondrial respiration [20, 21]. Despite ongoing efforts in the area of radiation proteomics, current database are too small to postulate strong conclusions concerning health consequences after space radiation exposures.

E. **Metabolomics**. Metabolomics is the high throughput molecular analytical approach that aims to identify and quantify concentration changes of all metabolites (i.e. small molecules) in cells, tissues, or biofluids such as serum and urine. While genomics, transcriptomics, and proteomics are relatively mature fields, metabolomics has now gained sufficient momentum and acceptance to join the ranks of the other "omic" approaches and adds another dimension to systems biology. However, our knowledge of the metabolome is still limited and represents an untapped resource for biomarker discovery, pathway analyses, and risk modeling. Although NMR-based studies helped develop the field of metabolomics, it has the tendency to detect highly abundant metabolites and ignore lower abundant metabolites [22]. Consequently, important low abundance metabolites that might contribute more significantly to the observed pathophysiologic states remained undiscovered. Over the last ten years, high- or ultra- performance liquid chromatography (UPLC) coupled with highly sensitive time-of-flight mass spectrometry (TOFMS, also referred to as QTOF) have provided the scientific community with unprecedented

capabilities in resolving thousands of metabolites typically less than 1 kilodalton (kDa) in size in a biological sample [22-29]. Most recent instrumentation systems can identify metabolites with errors in mass measurement below 5 parts per million leading to highly accurate mass measurement of metabolites. Such accuracy in mass measurement is invaluable for determining the identity and structure of novel metabolite biomarkers.

We have applied metabolomics to assess space radiation-induced changes in mouse intestines and cultured human colonocytes. Our study of the intestines of mice exposed to heavy ion (^{56}Fe) space radiation has shown a metabolite profile distinct not only from sham-irradiated control groups but also from low linear energy transfer (LET) γ radiation [30]. Importantly, analysis of ^{56}Fe-irradiated samples allowed us to identify molecules such as prostaglandin E2, which not only have biological relevance to intestinal pathologies in human but also have potential value in risk assessment (Figure 7). Integrated pathway analysis revealed that ^{56}Fe radiation induced selective upregulation of 'prostanoid biosynthesis' and 'eicosanoid signaling' that is linked to intestinal inflammation suggesting a higher risk of inflammatory responses in intestine during deep space missions. Additionally, higher perturbations in nucleotide and amino acid metabolism after ^{56}Fe compared to γ radiation was also evident [30].

Global metabolic profiling of cells, tissues, or organisms is useful in relating to changes at the other ‘omics’ level including genomics and epigenomics, and to identify health risk markers specific to exogenous or endogenous harmful agents. A myriad of cellular responses at the genomic, proteomic, as well as at the metabolomic level are expected after radiation exposure [7, 22, 31], and metabolomics has allowed identification of metabolites, which has the potential to serve as post-exposure risk-markers for long-term adverse sequela such as cancer. Metabolomics are increasingly used towards understanding radiation toxicity [22, 32-35] and

have the potential for assessing disease promotion and progression potential of space radiation exposure through tissue specific biomarker identification. Metabolomics offers the opportunity to assess changes at the small molecule level during injury responses including subtle changes in metabolism due to alterations by space radiation injury responses, which are expected to differ in important aspects from terrestrial γ radiation injury responses. Furthermore, with comprehensive 'omics'-based strategies we can assess changes at the genomic, epigenomic, and transcriptomic levels and link them with downstream changes at the proteomic and ultimately metabolomic levels. This will then provide the basis for a complete systems biology approach rather than just relying on a single level of "omics" analysis. The goal is to develop an 'omics'-based systems biology understanding of underlying pathways of toxicity, and then apply them to cellular, tissue, and organismal parameters for comprehensive space radiation risk model development.

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Figures and Legends

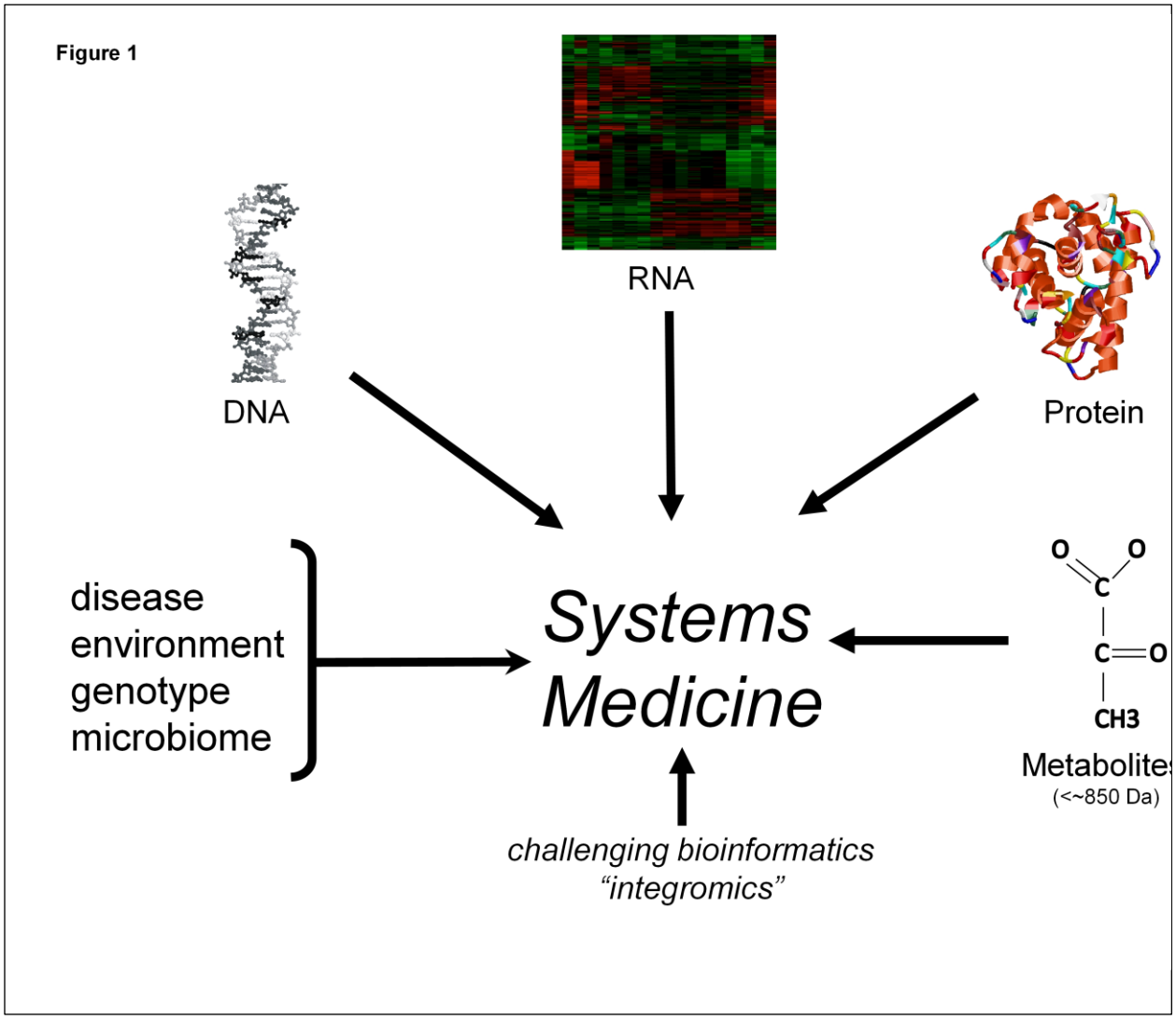


Figure 1. Schematic representation of levels of omics-based approaches available to analyze space radiation related risk estimates. Integrated omics-based systems biology databases could be employed to relate molecular, cellular, tissue, and physiological parameters for a comprehensive space radiation related health risk model development.

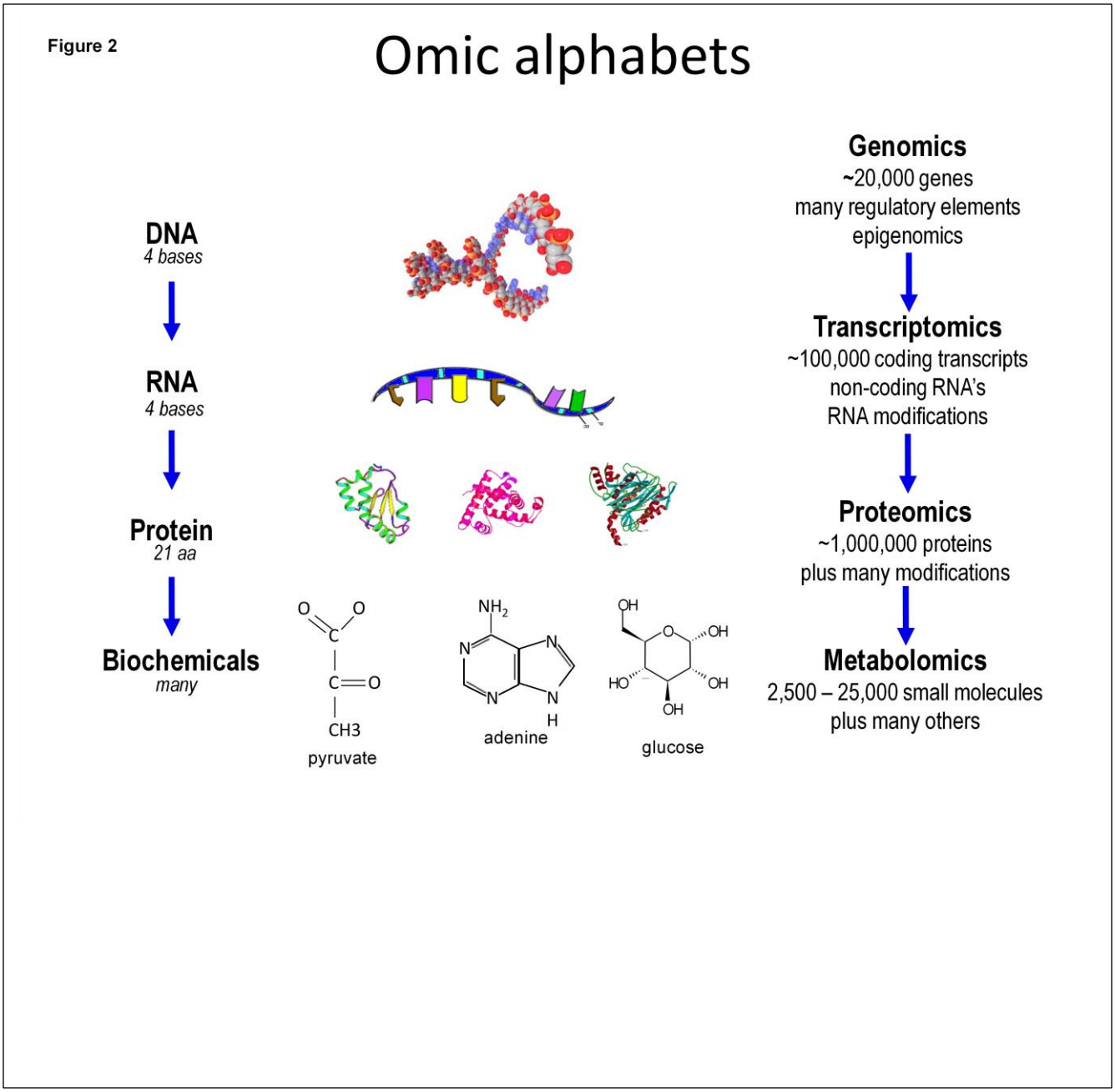


Figure 2. Schematic representation of major omics-based approaches available to develop omics signatures of space radiation-induced health risks. While information in cells is initiated in genome (DNA) and flows through transcriptome (mRNA), to proteome (protein), exposure to an exogenous stressor such as space radiation could result in a myriad of changes at different omics levels leading to alterations in the cellular metabolic profile (metabolome). Characterization of space radiation-induced changes at multiple omics level sould allow identification of better health risk markers, which will aid in reliable risk assessment.

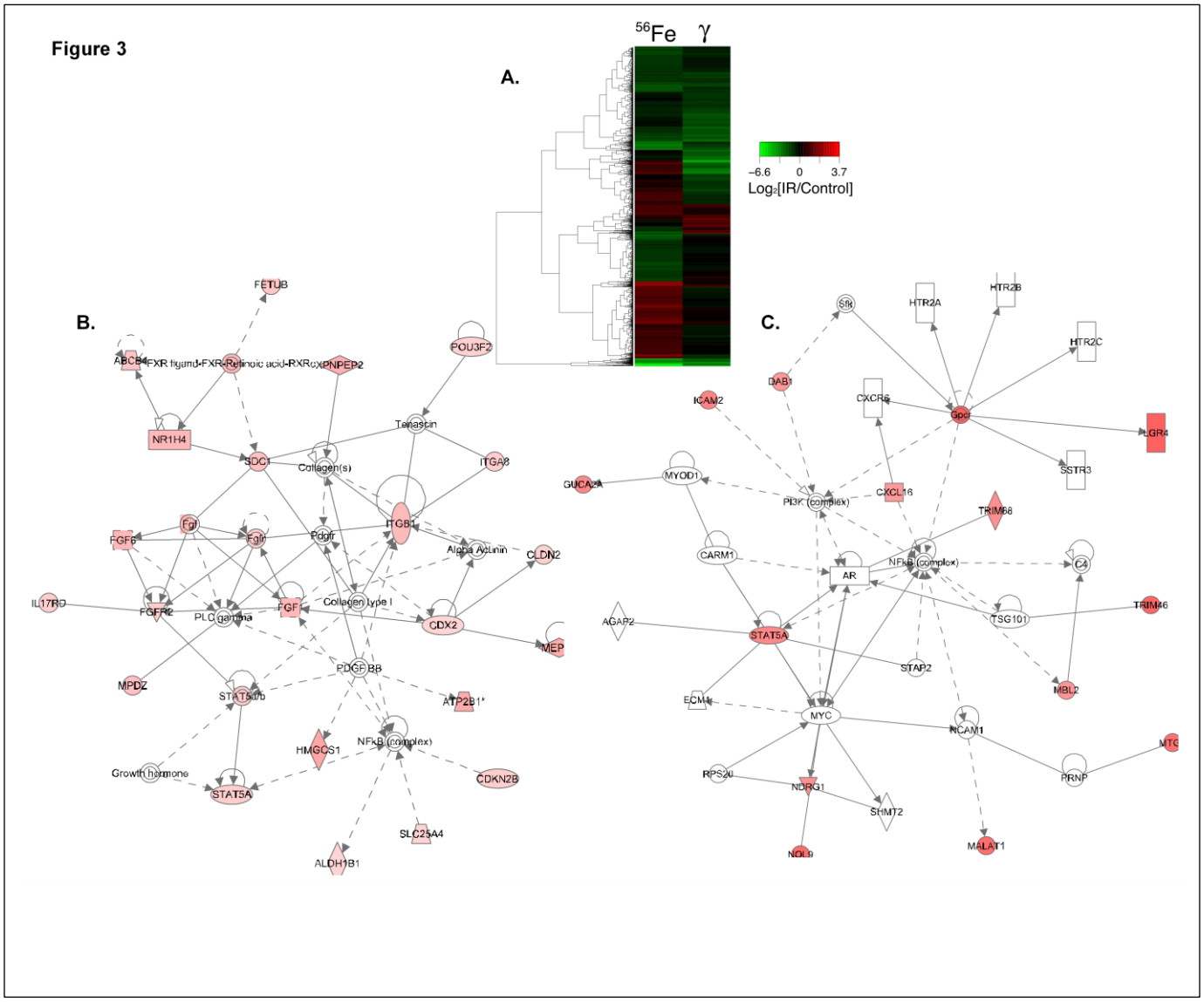


Figure 3. Differential alterations of intestinal transcriptomes 2 months after exposure to γ (7 Gy) and ^{56}Fe (6 Gy) radiation. A) Microarray data was normalized using GeneSpring GX (Agilent Technologies, Santa Clara, CA) as per instruction in the software manual. Such data normalization removes fluorescent intensity bias in the arrays and increases detection sensitivity and allows meaningful comparison of datasets among different experimental groups. In the current study normalized datasets were subsequently log transformed and differences of the mean log sham-irradiated control and mean log gamma or mean log. ^{56}Fe are presented as hierarchical clustering

map of fold changes along with the log2 scale ranging from -6.6 (green; downregulation) to +3.7 (red; upregulation). Hierarchical clustering is an unsupervised statistical tool to sequentially combine similar clusters until all objects in the same group produces a tree often called a dendogram that allows microarray data analysis based on similarity of features. The dendrograms provide qualitative means of assessing the similarity between genes and groups. Finally the combined features are presented as "heat map," where the color represents (red, green, and black) the expression level of the gene; red represents up-regulation, green indicates down regulation and black indicates unchanged expression of genes. B) Ingenuity Pathway Analysis (IPA, Ingenuity Systems Inc., Redwood City, CA, USA) was used for assignment of biological function as well as for identifying perturbed signal transduction networks associated with microarray datasets. IPA combines the uploaded data and the Ingenuity Pathways Knowledge Base (IPKB), created with information from available literature, to identify biological networks that are significantly over-represented in the gene expression data. For γ radiation (Figure 3B), the top signaling network identified in IPA show fibroblast growth factor 1 (FGF1) as the nodal molecule and FGF1 signaling has been demonstrated to be involved in mitogenic and angiogenic activity in different cell types. C) For ^{56}Fe radiation, the top upregulated signaling network identified in IPA showed genes related to NFκB signaling in mouse intestinal tissue 2 months after radiation exposure.

Figure 4

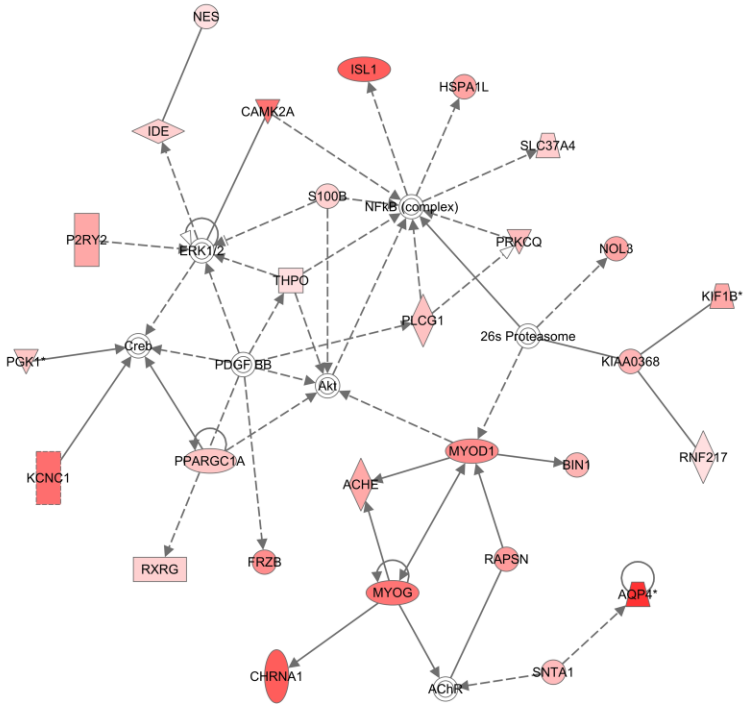


Figure 4. Ingenuity pathway analysis (IPA) of significantly perturbed mammary gland transcriptome 2 months after exposure to 2 Gy γ radiation identified a top signaling network with genes involved in carcinogenesis. The network can be associated with cellular growth and development with NFkB as the nodal molecule. At least 12 genes (ACHE, HSPA1L, MYOD1, NES, NOL3, P2RY2, PGK1, PLCG1, PPARGC1A, PRKCQ, S100B, and SNTA1) from this upregulated network are associated with initiation and progression of human cancer [36]. The intensity of red color reflects level of increased expression.

Figure 5

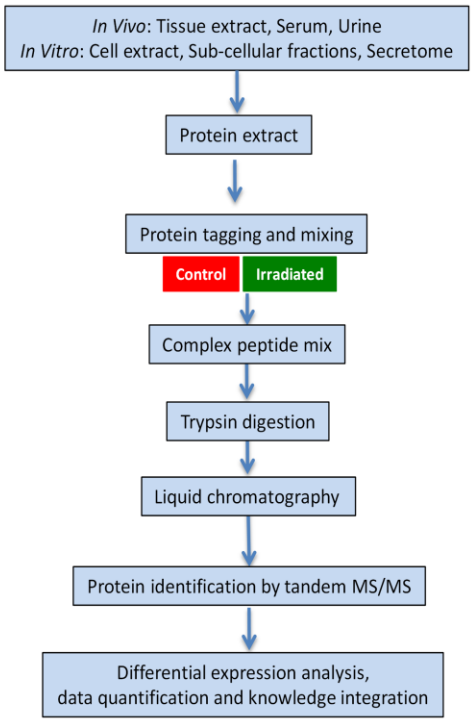


Figure 5. Mass spectrometry based proteomics approaches. Mass spectrometry based proteomics utilizes high resolution, liquid chromatography, followed by mass spectrometry-based selection and identification of differentially expressed proteins.

Figure 6

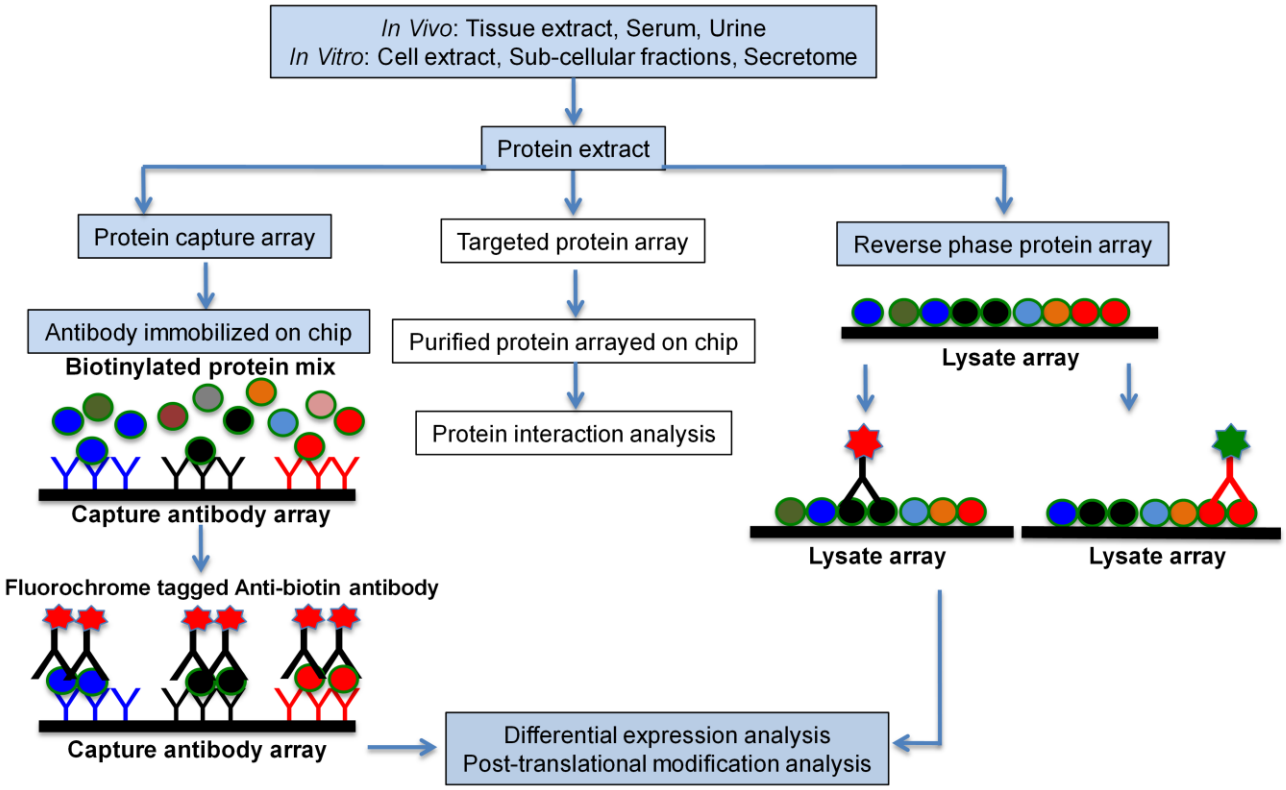
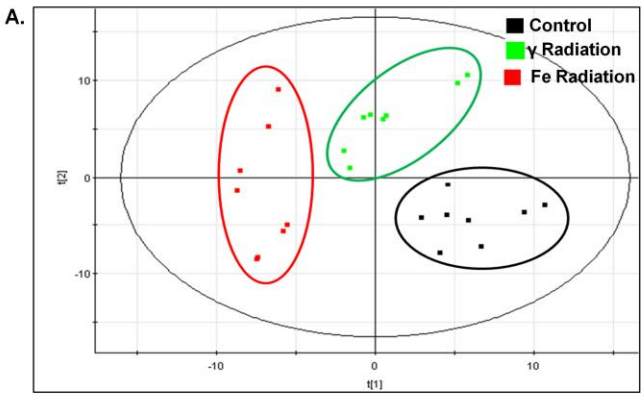
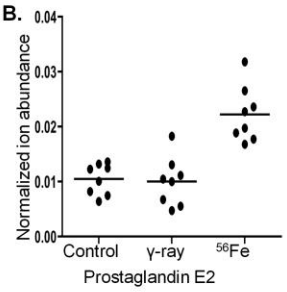
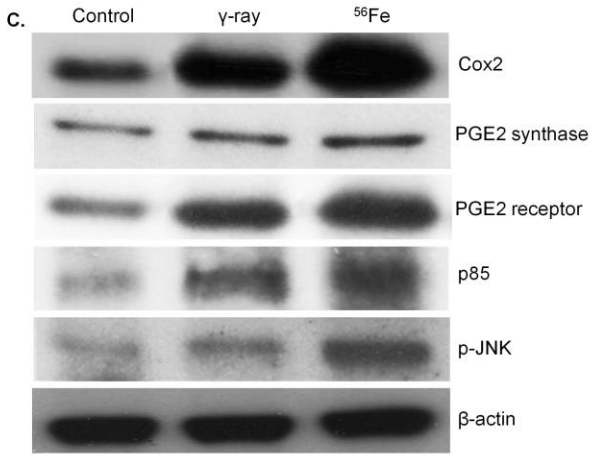


Figure 6. Protein capture array based proteomics approaches. Protein array based approaches include protein capture array, targeted protein arrays, and reverse phase protein array and are alternatively used for large scale global protein profiling.







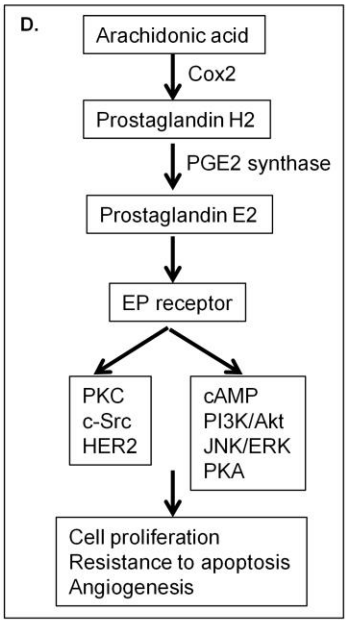


Figure 7

Figure 7. Multivariate analysis of mouse intestinal metabolomics and signaling pathway analysis obtained 2 months after sham, γ (2 Gy), and ^{56}Fe (1.6 Gy) radiation exposure shows distinct metabolic profiles and pathway alterations in negative ionization mode. A) Principal component analysis (PCA), an unsupervised large dataset analysis method, was used to determine similarities/differences among the metabolomics datasets of the experimental groups [37]. The PCA provides principal component (PC) scores where each score is representative of an

individual sample and allows plotting of multivariate datasets in two dimensions known as scores plot. The scores in the scores plot represent principal component 1 [t1] and 2 [t2] for each sample and clustering of scores denotes similarities and scores falling far apart in the plot suggests differences. Scores plot presented shows distinct class separation among the study groups. B) A selective biomarker, prostaglandin E2, differentially altered after exposure to two types of radiation is shown, C) Immunoblots showing increased levels of PGE2 synthase, PGE2 receptor, PI3K (p85), phospho-JNK, and Cox2 two months after ^{56}Fe radiation exposure. D) Greater activation of PGE2 dependent signaling pathways has the greater potential for increased proliferation of intestinal epithelial cell after ^{56}Fe radiation [30].