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LETTERS

Uncovering the potential of Fatty Acid Binding Proteins for predicting radiation-induced gastrointestinal injury

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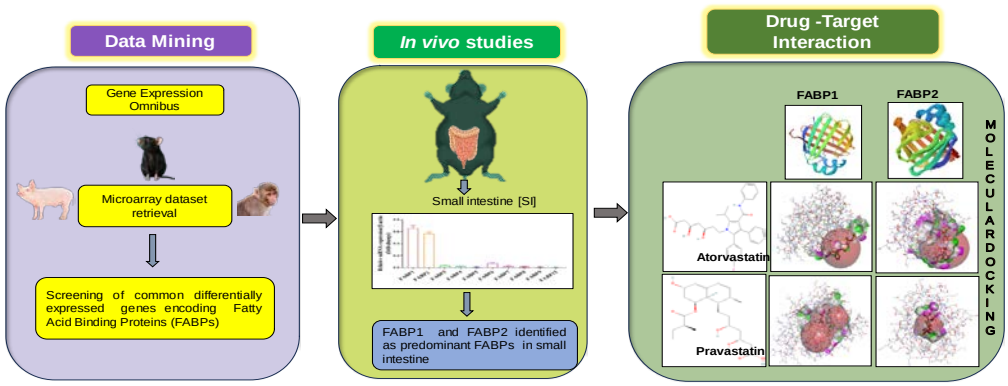
Article



ABSTRACT Gastrointestine (GI) depression is a severe complication identified in individuals after intentional or unintentional radiation exposure. Early detection of radiation-induced GI injury (RIGI) is important for medical management with supportive care, which can largely be achieved using organ specific biomarkers. Fatty Acid Binding Proteins (FABPs) are 14-15 kDa cytosolic proteins which are quickly released into circulation in the event of tissue injuries. The present investigation aims to unravel the potential of FABPs as candidate targets to predict RIGI. Utilizing data mining approach, FABP genes differentially expressed in 06 microarray datasets retrieved from Gene Expression Omnibus (GEO) database across distinct species were identified. The abundance of 10 genes encoding FABPs were checked in intestinal tissue of male and female C57Bl/6 mice by qRT-PCR analysis. FABP1 and FABP2 were identified as the abundant genes expressed in small intestine of both the sexes. In order to explore FABP1 and FABP2 as possible targets for radioprotection, we selected approved hydrophilic and lipophilic statins to perform molecular docking studies. The findings highlighted that FABP1 and FABP2, expressed in intestine, can act as potential biomarkers for RIGI as well as their drug targets can be explored as promising radiation countermeasure agents.

Keywords: Ionizing radiation, FABPs, biomarkers, gastrointestine, statins

INTRODUCTION

Cancer is unequivocally recognized as one of the leading causes of disease and mortality. Globally, there are about 19 million new instances of cancer diagnosed per year, and 10 million deaths are estimated. The use of radiotherapy is acknowledged as a crucial component in the treatment and management of cancer. Over the past century, radiotherapy treatment has contributed to substantial advancements that have improved patient outcomes.[1] However, therapeutic radiation exposure render patients susceptible to ionizing radiation (IR) induced injury, which often interferes with treatment strategies and outcomes. Further, accidental radiation exposure during radiological or nuclear accidents can also induce detrimental health effects, ranging from acute radiation sickness to chronic long-term risks like carcinogenesis and genetic damage. The deleterious biological effects of IR can manifest as severe tissue and organ dysfunction. Given the heightened radiosensitivity of the GI system to radiation and possibility of early mortality, the medical management of this organ is of paramount importance. The small intestine, a vital component of the GI tract, is involved in nutrient digestion and absorption. Its delicate epithelial lining and rapid cellular turnover render it highly susceptible to IR-mediated damage. Radiation exposure can inadvertently injure the small intestine, resulting in development of RIGI. Acute symptoms, such as nausea, vomiting, and abdominal pain, often manifest post radiation exposure.[2] Conversely, delayed symptoms, including diarrhea, recurrent abdominal pain,

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malabsorption of salts and fats, and bacterial overgrowth, can emerge months to years post-radiotherapy.[3,4]

Early detection of small intestinal damage is crucial for improving patient outcomes and survival. Although enterocyte depletion can be correlated with altered citrulline levels to predict GI injury, its relatively slow turnover rate (3.5-5 days) limits its utility for early diagnosis.[5,6] Consequently, the identification of early biomarkers is essential for the rapid diagnosis and medical management of individuals exposed to radiation. Data mining has emerged as a reliable approach for identifying organ-specific biomarkers associated with disease progression, predicting risk factors, and elucidating relevant pathways and interacting genes through the analysis of microarray datasets available on the GEO database.[7,8] This approach has also been demonstrated in the prediction of diseases.[9] Precise delineation of molecular pathways implicated in RIGI is essential for designing targeted therapeutic interventions. By uncovering the role of key proteins or signaling cascades involved in cellular responses to irradiation, critical targets for tissue damage and drug development processes can be identified. Such strategies hold the potential to mitigate radiation toxicity in both therapeutic and accidental exposure scenarios, ultimately improving treatment outcomes and patient health.

FABPs are low molecular-weight (14-15 kDa) intracellular proteins crucial for fatty acid metabolism.[10] They transport fatty acids and are key mediators in metabolic and inflammatory processes. Till date, ten different isoforms of FABPs have been identified and named as per the tissue in which these are localized and first identified.[11] Due to its tissue specificity, FABPs are considered as good candidates for organ specific biomarkers. They have been known to be involved in the development of various metabolic disorders and pathologies.[12]

In this study, we have used pre-existing six microarray datasets from the GEO database (https://www.ncbi.nlm.nih.gov/geo/), to identify different isoforms of FABPs present in datasets corresponding to small (*Mus musculus*) and large animals (*Sus scrofa domesticus* and *Macaca mulatta*). By analyzing the six microarray datasets (GSE104121, GSE173427, GSE102971, GSE143581, GSE141515, GSE182829), we identified and selected differentially expressed FABPs in whole blood and intestine. The abundance of FABP isoforms was checked in the small intestine of C57Bl/6 male and female mice. Molecular docking was performed to predict the binding affinity and orientation of potential drug candidates within the binding site of the target proteins.

EXPERIMENTAL PROCEDURES

Assembling microarray datasets

Six microarray datasets were downloaded from the GEO database a public repository for gene expression data. The keywords used for searching were "Radiation", "GI injury" and "Blood". Three datasets (GSE104121, GSE173427, GSE102971) corresponded to whole blood samples of *Mus musculus* (mice), *Sus scrofa domesticus* (domestic pig), and *Macaca mulatta* (rhesus macaque). The remaining three datasets (GSE143581, GSE141515, GSE182829) were derived from small intestine samples of *Mus musculus* (mice) and *Sus scrofa domesticus* (domestic pig).

Differential FABP gene expression analysis

We employed the GEO2R tool (http://www.ncbi.nlm.nih.gov/geo/) to identify DEGs and select differentially expressed FABP genes in all six selected microarray datasets (GSE104121, GSE173427, GSE102971, GSE143581, GSE141515, GSE182829). Selection criteria for the genes included |log2 (fold-change)|> 1.0 and p-value < 0.05.

Venn diagram and heatmap construction: Graphical depiction and visualization of FABP genes across multiple microarray datasets

Different FABP isoforms were found differentially expressed in variable microarray datasets retrieved from GEO database. The FABP genes commonly and exclusively expressed in all the microarray datasets of intestine as well as blood samples were shortlisted and depicted using venn diagrams. Additionally, those FABP genes commonly expressed between intestinal and blood microarray datasets were also represented through venn diagram. Further, heatmaps showing the differentially expression pattern of FABP genes in each microarray dataset was constructed.

Animal experimentation

The control male and female C57Bl/6 mice aged 10–12 weeks were obtained from Institute's Animal Experimental Facility. All the animals were maintained under controlled environmental conditions including temperature (26 ± 2°C), humidity (50-70%) and a 12-hour light/dark cycle. The animals were acclimatized for 2 – 4 days prior to the experimentation. All the experimental work was conducted in accordance with protocols approved in The Institutional Animal Care and Use Committee (IACUC), via approval number INM/IAEC/2022/10 dt. 23.01.23.

Tissue harvesting, total RNA isolation and quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) analysis

30 mg small intestine tissues from control male and female mice were harvested for total RNA isolation using RNeasy Mini Kit (Cat. No. 74104 Qaigen, Hilden, Germany). The total RNA was processed for cDNA synthesis using iScript™ cDNA Synthesis Kit (Cat. No. 1708891, BIO-RAD California, USA) as per manufacturer's protocol. iTaq™ Universal SYBR® Green Supermix (Cat. No. 1725121, BIO-RAD, California, USA) was used for performing qRT-PCR and reactions were analyzed on the IVD CFX Real- Time PCR System (Bio-Rad Laboratories India Pvt. Ltd., Hercules, California, USA). The oligonucleotide sequence pair used for amplification of each gene is presented in Table 1. After 40 cycles of amplification, the fold change in the transcript level of each group was calculated based on ΔΔCT method. The housekeeping gene, β-Actin was utilized to normalize the mRNA expression levels of each gene and the relative expression level was represented as fold change.

Molecular Docking Studies

Preparation of ligand

The 3D structures of "Atorvastatin" (PubChem ID:60823) and Pravastatin (PubChem ID:54687) were downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in Structural Data File (SDF) format. Energy minimization of the

Atorvastatin and Pravastatin structures was performed using AutoDock, a molecular docking software (https://autodock.scripps.edu/). The optimized structures of Atorvastatin and Pravastatin were converted into Protein data Bank, partial charge and atom type (PDBQT) file format using Open Babel software (https://sourceforge.net/projects/openbabel/).

Preparation of protein

The 3D structures of human FABP1 (PDB ID:3STK) and FABP2 (PDB ID:1KZW) were downloaded from the Protein Data Bank (PDB) in PDB format. Similarly, the 3D structure of mice FABP1 and FABP2 proteins were downloaded with PBD ID AF-P12710-F1 and AF-P55050-F1, respectively (https://www.rcsb.org/). Protein preparation was done in Discovery Studio Biovia (https://discover.3ds.com/discovery-studio-visualizer-download). For protein preparation, water and heteroatoms were removed, and uniform polar hydrogens were added. The Gasteiger charge was calculated, and the prepared protein was saved in PDB format.

Table 1. Primer sequences of genes encoding different fatty acid binding proteins used in qRT-PCR.

Sl. No	Gene name	Oligo sequence	
1	FABP1	FP	GAAAATCAAACTCACCATCACC
		RP	CTGCCTTGACTTTTTCCCC
2	FABP2	FP	TGGACCATTGAGGGAAATAAAC
		RP	GCTGATAGGATGACGAATGAG
3	FABP3	FP	ACCAAGCCTACTACCATCATC
		RP	ACGCCTCCTTCTCATAAGTC
4	FABP4	FP	AGCTTGTCTCCAGTGAAAAC
		RP	ATAACACATTCCTTCACCTTCC
5	FABP5	FP	TGCTTTTGTGCTCTCCCTC
		RP	CCGTCTCAGTTTTTCTGCC
6	FABP6	FP	CAAAGAATGTGAAATGCAGACC
		RP	TCTCCACCAACTTGTCACC
7	FABP7	FP	TGAAGAAACCAGCATAGATGAC
		RP	TCATAACAGCGAACAGCAAC
8	FABP8	FP	AGAAGTGGGATGGGAAAGAG
		RP	GAAGAAACCAGACAAGCCAG
9	FABP9	FP	GTGGATGGAAAAATGGTAGTGG
		RP	GGCAAGTTCTTTGTGGTGG
10	FABP12	FP	AGATGGGAAAATGGTGGTGG
		RP	GGATGACAATTTGAGAGCATGG
11	β-Actin	FP	TGTTTGAGACCTTCAACACC
		RP	ATGTCACGCACGATTTCC

FP: Forward primer, RP: Reverse primer

Ligand and protein interaction

The molecular docking study was performed using the AutoDock Vina plugin within PyRx (https://pyrx.sourceforge.io/) software. The prepared proteins were uploaded and designated as the macromolecule, while the ligands (atorvastatin and pravastatin) were uploaded and converted into PDBQT format. A grid box (X=53.6822, Y=44.0567, Z=46.7644) was made around the protein-ligand complex, and the ligand was subjected to exhaustiveness of 8 in a single run for docking study. Other docking parameters were kept as default. Docked structures were visualized in discovery studio Biovia.

Statistical analysis

The acquired experimental data is examined and shown as mean ± SD from two separate studies, each including four mice. One-way analysis of variance using the Newman-Keuls multiple comparison test (V, 5.01; GraphPad Prism, San Diego, CA, USA; https://www.graphpad.com) was used to determine the statistical difference between the experimental groups. A p value < 0.05 was considered as threshold for statistical significance. For bioinformatics analysis, selection of DEGs was based on $|\log2 (\text{fold-change})| > 1.0$ and p-value < 0.05.

RESULTS

Identification of differentially expressed FABP genes

We selected six microarray datasets belonging to small and large animals - mice, domestic pig and rhesus macaque by utilizing the keywords “radiation", "GI injury and “blood”. Based on selection criteria of $p < 0.05$ and $|\log2 (\text{fold-change})| > 1.0$, we first identified total, upregulated and downregulated DEGs in respective datasets. The total genes, upregulated as well as downregulated DEGs in each dataset corresponding to intestine and blood are compiled in Table 2.

Table 2. Total, upregulated and downregulated differentially expressed genes (DEGs) in each microarray dataset.

GSE ID	Total DEGs	Upregulated DEGs	Downregulated DEGs
GSE104121	7824	3350	4474
	2221	1124	1097
	13458	4034	9424
	1953	1147	806
GSE173427	6952	997	5955
	11865	2340	9525
	7196	1516	5680
	10287	962	9325
	17788	2577	15211
	12610	3252	9358
GSE102971	13142	562	12580
	8623	386	8237
GSE143581	7910	2942	4968
GSE182829	2218	1010	1208
GSE141515	6531	22	6509
	4913	88	4825
	2205	25	2180

Selection of common FABP genes across different datasets

Various FABP isoforms were found expressed in microarray datasets belonging to different species. The heatmaps summarizing the gene expression patterns of differentially expressed FABP genes for blood and intestinal datasets are

presented in (Figure 1 a and b). Further, the exclusive and common differentially expressed FABP genes within and between blood and intestinal datasets were shortlisted. FABP1 and FABP2 were found commonly expressed between the three intestinal microarray datasets-GSE143581, GSE182829 and GSE141515. FABP6 was found commonly expressed between GSE143581 and GSE182829 whereas FABP5 was identified common between GSE143581 and GSE141515. Further, FABP4 was exclusively observed in GSE143581 (Figure 1c). Further, FABP5 was commonly expressed between all the three datasets corresponding to blood (GSE104121, GSE173427 and GSE102971). Three genes namely, FABP3, FABP4 and FABP7 were common between GSE104121 and GSE173427. FABP9 was exclusive to GSE104121 whereas FABP1, FABP2 and FABP6 were exclusive to GSE173427 (Figure 1 d). Notably, FABP1, FABP2, FABP4, FABP5, and FABP6 were found commonly expressed between intestinal and blood microarray datasets. And, FABP3 and FABP7 were separately found in all the blood microarray datasets. (Figure 1 e).

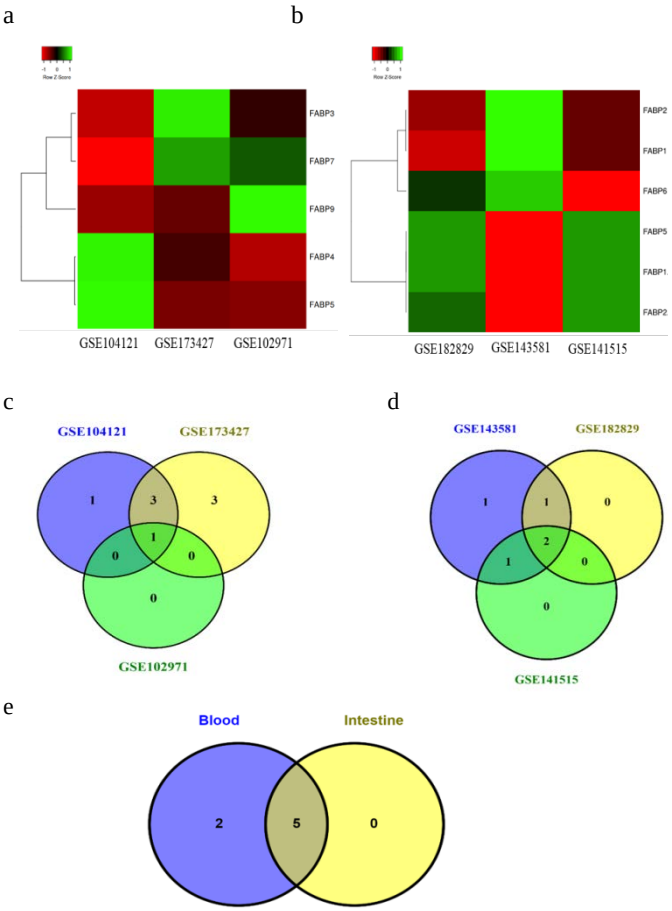

Figure 1. Identification of differentially expressed genes (DEGs) encoding FABPs in different microarray datasets. Heatmaps of microarray datasets corresponding to (a) blood and (b) intestine. Each column represents a microarray dataset whereas each row indicates expression values of FABP gene. Red indicates downregulated genes and green corresponds to upregulated genes. Venn diagrams representing common and exclusive FABP genes within microarray datasets corresponding to (c) small intestine, (d) blood and (e) common to blood and intestine.

qRT-PCR analysis: Analysis of predominant FABPs in mice intestine

We were further interested in checking the abundance of all FABP isoforms in small intestine of control C57Bl/6 male and female mice. The expression of a total of 10 FABP isoforms was checked at mRNA level. qRT-PCR analysis revealed that FABP1 and FABP2 were predominantly expressed FABPs in male mice. Whereas, high expression of FABP1, FABP2 and FABP6 was found in intestine of female mice. The remaining FABP transcripts, FABP3, FABP4, FABP5, FABP7, FABP8, FABP9 and FABP12 did not display significant expression in both male and female small intestine (Figure 2 a and b).

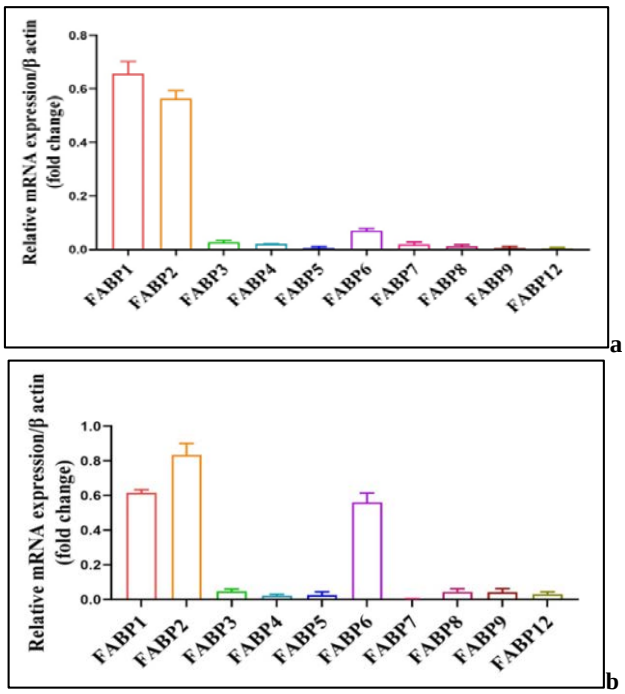

Figure 2. Assessment of predominant FABPs isoforms expressed in small intestine of male (a) and female mice (b). The relative expression of each gene was represented based on fold change after normalization against housekeeping gene β-Actin.

Molecular Docking studies: Prediction of the potential of FABP1 and FABP2 as targets for radioprotection

FABP1 and FABP2 were identified as critical targets to predict radiation-induced injury to the intestine as these were commonly expressed in all 06 selected microarray datasets as well as were found most abundant in the mice intestine. Since FABPs play important role in lipid metabolism, we further explored these proteins as possible targets for radioprotection. Therefore, we employed a molecular docking approach to predict the interactions between FABP1 and FABP2 with US-FDA approved cholesterol-lowering drugs-lipophilic, atorvastatin and hydrophilic, pravastatin. The details of each protein and the ligand including the binding affinity and specific sites of the protein-ligand complex are provided in Table 3.

Table 3. Binding energies and interacting amino acid residues involved in the ligand–protein interaction of the selected ligands against FABP1 and FABP2 proteins of mice and human origin.

Species	Protein	PDB ID	Ligand	Binding Affinity (kca/mol)	Interacting amino acid residues
Mice	FABP1	AF-P12710-F1	Pravastatin	-5.5	PRO17, GLU16, PHE15, GLU13,LYS33, ASN14, GLN12, SER11, ASP34, LEU9, GLN10
	FABP2	AF-P55050-F1		-5.4	GLY87, PHE3, ALA2, MET1, GLN43, PHE46, ILE41, ILE85
	FABP1	AF-P12710-F1	Atorvastatin	-6.1	GLN12, ASN14, PHE15, GLU16,PRO17, LYS33
	FABP2	AF-P55050-F1		-5.9	GLU64, GLY45, PHE63, THR49, ASP44, VAL50, VAL62, LYS47, PHE48, THR42, ILE41, PHE3, META1, ASN65, GLN43, ILE35, ALA2, LEU90, ASN38, LYS39, GLY37
Human	FABP1	3Stk	Pravastatin	-5.2	GLU101, PHE93, ASN103, LYS94, LYS92, THR81, GLY80, ARG79, THR67
	FABP2	1kzw		-8.9	VAL83, SER100, THR93, THR73, PHE95, MET113, MET74, LEU9, ILE123, THR110, ILE 109, SER124, SER39, TYR7, ASN111, ARG122, ILE52, THR102, PHE63, PHE48, LEU91, ILE41, LEU71, PHE50
	FABP1	3Stk	Atorvastatin	-5.8	PHE62, ASN71, ILE58
	FABP2	1kzw		-6.4	GLU67, GLU68, LYS80, CYS69, GLU62, GLU70, PHE63, HIS47, PHE48, LYS49, THR64

Interactions of these drugs were evaluated with FABP1 and FABP2 of mouse and human origins. The human FABP1 protein interacts with pravastatin by binding through variety of amino acids as GLU101, PHE93, ASN103, LYS94, LYS92, THR81, GLY80, ARG79 and THR67 with a B.E. (binding energy) of 5.2 kcal/mol (Figure 3 a).

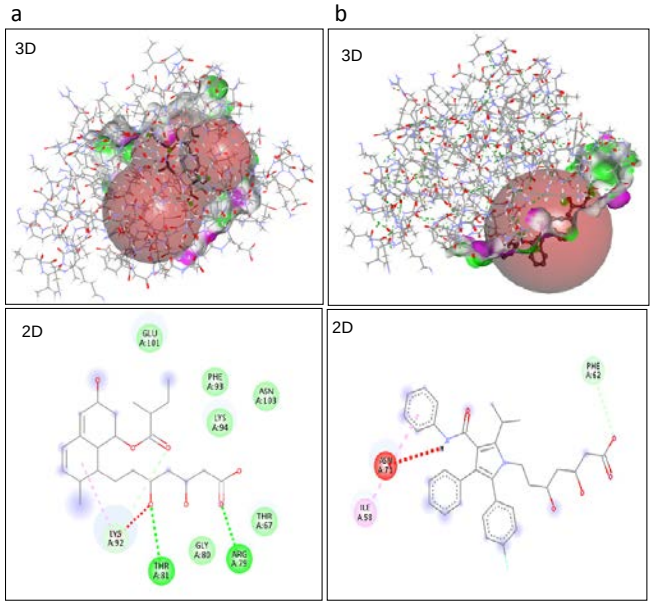

Figure 3. Molecular docking studies displaying interaction of human FABP1 protein with pravastatin and atorvastatin. PubChem database was employed to retrieve the 2D and 3D images of the ligand. (a) 3D and 2D image displaying human FABP1 interaction with pravastatin through binding to various amino acid residues with binding energy of -5.2 kcal/mol. (b) 3D and 2D images showing human FABP1 interaction with atorvastatin through few amino residues and binding energy of -5.8 kcal/mol. The details of the interacting amino acid residues are given in the text.

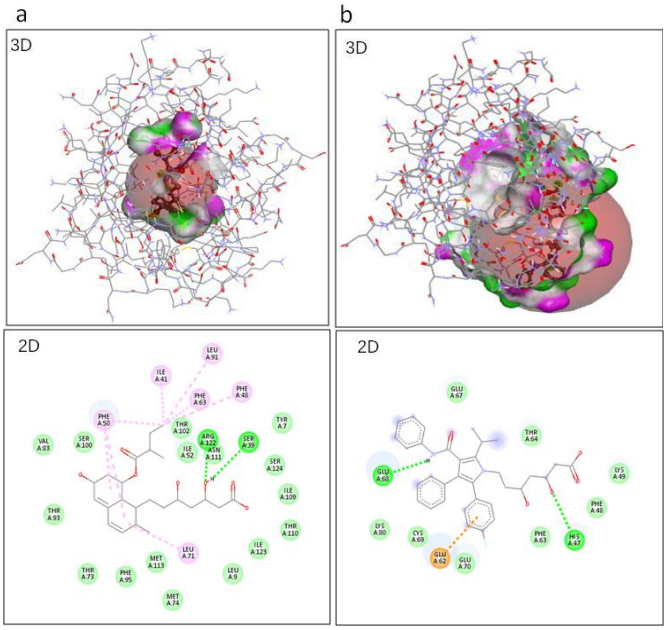

Figure 4. Molecular docking studies showcasing interaction of human FABP2 protein with pravastatin and atorvastatin. PubChem database was employed to retrieve the 2D and 3D images of the ligand. (a) FABP2 - Pravastatin interaction complex indicating the binding through variety of amino acid residues with binding energy of -8.9 kcal/mol (b) FABP2 interacts with atorvastatin through different amino acids residues and displayed binding energy of -6.4 kcal/mol. The details of the interacting amino acid residues are given in the text.

On the other hand, human FABP1-atorvastatin complex displays B.E. of -5.8 kcal/mol through formation of bonds with three residues - PHE62, ASN71 and ILE58 (Figure 3 b). Similarly, human FABP2 protein is predicted to bind with

pravastatin through VAL83, SER100, THR93, THR73, PHE95, MET113, MET74, LEU9, ILE123, THR110, ILE 109, SER124, SER39, TYR7, ASN111, ARG122, ILE52, THR102, PHE63, PHE48, LEU91, ILE41, LEU71 and PHE50 with a B.E. of -8.9 kcal/mol (Figure 4 a).

Human FABP2 binds with atorvastatin through GLU67, GLU68, LYS80, CYS69, GLU62, GLU70, PHE63, HIS47, PHE48, LYS49 and THR64 with B.E. of -6.4 kcal/mol (Figure 4 b). Additionally, mice FABP1 and FABP2 proteins were also docked with both statins. The interaction complexes of mice FABP1 and FABP2 proteins each with pravastatin and atorvastatin are provided in Figure S1 and S2. The binding affinity and the interacting amino acid residues of the protein-ligand complexes are displayed in Table 3.

DISCUSSION

Cancer represents a significant burden of disease at a global level. Worldwide, a major section of people received cancer diagnosis each year, and over half of those individuals pass away as a result of the disease. It is the second most common cause of mortality in many nations.[13] A growing number of medical professionals are assisting long-term survivors who experience a range of persistent side effects linked to their radiation treatments. The most common adverse effect of pelvic/abdominal radiation therapy is radiation-induced GI damage. This often interferes with continuation of treatment regime and is a major contributor of mortality among radiation exposed victims.[14,15] The only reliable biomarker recommended for evaluating GI injuries following radiation exposure is plasma/urine citrulline. Enterocyte depletion is a prerequisite for the detection of citrulline levels in biofluids. The turnover rate of 3.5-5 days limits its application for early detection of GI damage.[5,6] Consequently, the discovery of early biomarkers is essential for an early diagnosis and medical management of victims exposed to radiation. Data mining has emerged as a cornerstone in biomedical research, particularly in the realm of biomarker discovery and understanding of mechanism related to disease progression. Its application in analyzing freely available microarray datasets on platforms like GEO has made possible the analysis of large data retrieved from different subjects to identify and infer complex relationships involved in variety of biological pathways.[7,9]

In our previous study, through data mining approach, we have found lipid metabolism as one of the enriched pathways along with other pathways in KEGG analysis. Further, based on a detailed expression analysis of selected hub genes associated with lipid metabolism in irradiated intestine, FABP genes were found to be significantly regulated in mice intestine in response to radiation. Therefore, in the present study, we were interested to check the expression profile of FABPs in microarray datasets across the species to unravel their role as a candidate targets for prediction of radiation induced injury to intestine.

FABPs belong to a family of cytoplasmic proteins which perform significant functions in the metabolism and transportation of fatty acids as well as assist in maintenance of lipid homeostasis in different tissues.[10] Ten isoforms of FABPs are reported which are expressed in variety of tissues. Based on the organs or tissues where they were first discovered, isolated, and found to be most prevalent, the isoforms are named likewise.[10,16] The FABPs family consists of ten isoforms namely - L-FABP/FABP1(liver), I-FABP/FABP2 (intestinal), H-FABP/FABP3 (heart), A-FABP/FABP4 (adipocyte), E-FABP/FABP5/(epidermal), IL-FABP/FABP6 (ileal), B-FABP/FABP7 (brain), M-FABP/FABP8 (myelin), T-FABP/FABP9 (testis) and FABP12 (retina).[11] Due to its tissue specificity, FABPs represent suitable candidates for exploration as organ- specific biomarkers. They have the potential to serve as reliable surrogate markers, effectively replacing clinical endpoints in clinical trials or practice which could enhance diagnosis and monitoring, forecast relapses or response to treatment, and aid in the creation of customized medication therapies.[17,18] Since, FABPs are small proteins (14-15 kDa), they are quickly released into the systemic circulation and undergo clearance by the kidneys,[10] hence can suitably be measured easily in body fluids.[19]

Radiation is well known to cause GI injury at higher doses. The damage caused by radiation includes inflammation of intestinal epithelium, epithelial cell death, atrophy of villi, crypt shortening, diminished epithelial cell renewal which ultimately results in a denuded intestinal mucosal barrier. A compromised mucosal barrier leads to a loss of nutrients, water, and electrolytes, enhanced permeability to bacteria and antigens, sepsis, inflammation and organ dysfunction thus disrupting the intestinal homeostasis.[20,21] Many studies have highlighted the possibility of FABPs to evaluate the severity of enterocyte damage and correlated it with various GI pathologies.[19,22,23,24]

In the present study, we have utilized data mining approach to underline whether FABPs represent potential targets capable of assessing RIGI in different animal species. Six microarray datasets were selected and retrieved from GEO database out of which three corresponded to intestine tissue and three belonged to blood. The intestine specific datasets included - GSE143581 (mice), GSE182829 (minipig) and GSE141515 (mice). The blood specific datasets included – GSE104121 (mice), GSE173427 (minipig) and GSE102971 (rhesus macaque). The GSE143581 incorporated small intestine samples isolated from irradiated (10 Gy) mice whereas GSE182829 comprised samples from minipig small intestine treated with 15 Gy abdominal irradiation. The GSE141515 included mouse small intestinal organoids samples irradiated with 6 Gy of gamma rays at time points, 24h, 48 h and 96 h. Further, GSE104121 contained whole blood samples of mice exposed to 1, 2, 4, 8, and 12 Gy of gamma-rays at different time points (16 h, 24 h, and 48 h). The GSE182829 included whole blood samples harvested at different time points (day 1, 3 and 7) post total-body gamma irradiation of minipig with variable doses (1.7, 1.9, 2.1 and 2.3 Gy). On the contrary, GSE102971 contained ex vivo-irradiated peripheral blood samples isolated from rhesus macaques exposed to 0, 2, 5, 6, and 7 Gy at 24 h. In radiobiology, animal models are essential for elucidating the associated mechanisms as well as in the identification and validation of organ-specific biomarkers. The Animal Efficacy Rule enlists the guidelines for the development

of drugs for which human studies cannot be conducted due to ethical reasons. Therefore, simulation of radiation-induced damage by utilizing well characterized animal models with relevant end–points form the foundation of biomarker discovery as well as drug efficacy evaluation.[25] Hence, microarray datasets belonging to different species were selected to identify the common FABP targets indicative of radiation-induced intestinal injury. The total genes, upregulated and downregulated DEGs in each microarray datasets were identified. Thereafter, we shortlisted various FABP genes differentially expressed in all the six microarray datasets corresponding to intestine as well as whole blood samples treated with radiation. Further, the heatmaps were constructed to visualize the expression of differentially expressed FABP genes in the intestine as well as whole blood datasets (Figure 1 a and b). Heat maps are commonly used to illustrate a range of values coded by appropriate colour to highlight the gene expression pattern of multivariate data. They are frequently used to show patterns of gene expression in various samples or under different experimental conditions.[26] The same FABP genes did not display a uniform expression pattern across the four species indicating their differential regulation across different species. FABPs are expressed in a wide variety of species such as *Drosophila melanogaster*, *Caenorhabditis elegans*, *Mus musculus*, *Homo sapiens* and display evolutionary conservation.[12] Thereafter the exclusive and common FABP genes observed across different microarray datasets corresponding to small as well as large animals were enlisted. These genes were depicted in the form of Venn diagram, which enable comparisons between various experimental setups and techniques.[27] It was observed that FABP1 and FABP2 were consistently expressed in three microarray datasets corresponding to irradiated intestine (GSE141515, GSE182829 and GSE143581). On the other hand, FABP5 was identified as common DEG in all the three blood microarray dataset (GSE104121, GSE 173427 and GSE102971) (Figure 1c and d). Different FABPs are expressed in various tissues and upon radiation-induced damage could be released from any part of body. Therefore, different FABP genes were observed across whole blood microarray datasets. On the contrary, FABP1 and FABP2 isoforms are reported to be expressed in small intestine indicating their tissue specificity[11] and they were observed consistently expressed in intestinal microarray datasets. Thereafter, we were interested to identify the common FABPs expressed in intestine as well as blood in order to highlight the potential of these genes as tissue–specific potential targets which could be used to predict RIGI. Notably, FABP1, FABP2, FABP4, FABP5 and FABP6 were found commonly expressed between intestinal and blood microarray datasets (Figure 1 e). Further, the relative expression of the different FABPs was assessed in both male and female mice intestine. The qRT-PCR analysis revealed that FABP1 and FABP2 transcripts were predominantly expressed in male mice intestine. On the other hand, the intestine of female mice displayed higher expression of FABP1, FABP2 and FABP6 at mRNA level (Figure 2 a and b). It is well documented that intestine expresses three FABPs namely – FABP1, FABP2 and FABP6. FABP genes are reported as sexually dimorphic genes as targeted ablation of any of these three genes leads in sexually dimorphic phenotypes.[28] FABP1, often referred to as Liver-FABP (L-FABP) was initially discovered in the liver. However, it is also located throughout the small intestine but highly expressed in duodenum and jejunum. It binds to lipid species such as lysophospholipids, monoacylglycerols, fatty acyl CoAs, and prostaglandins. FABP2 also known as Intestinal FABP (I-FABP) is found exclusively in small intestine with maximum abundance in the jejunum region of small intestine. It uptakes fatty acids and mediates lipid trafficking in intestine. Moreover, FABP6 also referred to as Ileal Bile Acid Binding Protein (I-BABP) is majorly found in the distal region of the small intestine where it interacts preferentially with bile acids and maintains enterohepatic bile acid metabolism.[12,29] The small intestine is extensively involved in absorption and assimilation of fat which is required to meet the energy demands of the body. It absorbs lipids which is partly derived exogenously (from the diet) as well as endogenously (from enterocytes and bile). Enterocytes play a vital role in the production and delivery of lipid entities such as lipoproteins and chylomicrons into the lymphatic system, as well as in the absorption and use of fatty acids from the food. This process is mediated by an intricate series of various enzymes and transporters in the intestine. FABPs function to maintain lipid metabolic processes in the intestine and facilitate processes, such as digestion, uptake and re-synthesis of intestinal lipids as well as their packaging into pre-chylomicrons followed by their release into the lamina propria and finally to lymph.[30,31] Based on the results, FABP were identified as crucial targets modulated upon radiation exposure and represent good candidates for an intestine specific biomarker which can be measured in blood upon its release due to epithelium damage to radiation.

In our previous study, we have performed serum lipid profile analysis and found extensive alteration in cholesterol and triglycerides metabolism in radiation exposure. Also, FABP1 and FABP2 were identified as hub genes in intestine in radiation exposure (data communicated elsewhere). Further, it was well reported that cholesterol metabolism is extensively altered in radiation exposure as seen in radiotherapy patients and is managed with the use of cholesterol-lowering agents - statins.[32,33,34] Statins are prevalently used in the management of hypercholesterolemia and display pleiotropic effects as antioxidant, anti-inflammatory and antithrombotic effects. These are 3-hydroxy-3-methylgutaryl-coenzyme A (HMG-CoA) reductase inhibitors. HMG-CoA catalyzes the conversion of HMG-CoA to the mevalonate in the cholesterol biosynthesis pathway.[35] Statins are implicated in protection of normal cells from devastating effects of radiation.[36-39] Further, they have been demonstrated to prevent radiotherapy-induced fibrosis as well as inflammation in cancers. They are also reported to inhibit endothelial damage thereby alleviating radiation proctitis and improve survival of patients receiving radiotherapy for non-small cell lung cancer (NSCLC).[40-42] Therefore, we selected two FDA approved cholesterol lowering drugs namely, atorvastatin (lipophilic) and pravastatin (hydrophilic) and studied their interactions with FABP1 and FABP2 proteins. The molecular

docking studies suggested that FABP1 and FABP2 proteins from mice and human showed strong molecular interactions with atorvastatin and pravastatin. The human FABP1 protein interacts with pravastatin and atorvastatin by formation of strong bonds with many amino acid residues at various positions and displays binding affinity of -5.2 kcal/mol and -5.8 kcal/mol (Figure 3 a and b). Similarly, binding energies of -8.9 Kcal/mol and -6.4 Kcal/mol were observed with interaction of human FABP2 with pravastatin and atorvastatin respectively through variable amino acid residues (Figure 4 a and b). Therefore, docking studies revealed that FABP1 and FABP2 proteins could serve as potential therapeutic targets for radioprotection and can be explored for development of radiation countermeasures to prevent RIGI.

CONCLUSION

Radiation exposure whether planned (radiotherapy) or unplanned (nuclear accidents) cause damage to normal tissues resulting in a wide range of devastating effects in humans. RIGI is one of the side effects prevalently seen in individuals exposed to radiation unintentionally during nuclear catastrophes as well as for various cancer treatment modalities. The lack of organ-specific biomarkers for RIGI at an early stage poses a major challenge in the prediction, diagnosis and extension of supportive care to such individuals. Therefore, the present study aimed to explore the potential of FABPs as candidate early biomarkers to predict RIGI. Differentially expressed FABP genes were screened in each microarray dataset pertaining to irradiated intestine tissue and blood across different species. FABPs were identified as critical targets regulated by radiation in mice, minipig and monkey. However, male and female mice intestine displayed the expression of majorly two FABP isoforms namely-FABP1 and FABP2. Further, FABP1 and FABP2 could serve as candidate targets which can be explored for their radioprotective potential as revealed by molecular docking studies. However, a detailed analysis including protein expression studies as well as estimation of these FABPs in systemic circulation in mice model showing RIGI will facilitate an in depth understanding on regulation of FABP genes in radiation exposure.

SUPPLEMENTARY INFORMATION

The supplementary file contains the binding interaction complexes of mice FABP1 and FABP2 proteins each with pravastatin and atorvastatin (Figure S1 and S2).

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CONFLICT OF INTEREST

The authors hereby declare no conflict of interest in the present study. All authors have actively contributed and granted their approval for the submitted version of this article.

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