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The Human Salivary Proteome is Radiation Responsive

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Abstract

In the event of a nuclear incident in a heavily populated area, the surge in demand for medical evaluation will likely overwhelm our emergency care system, compromising our ability to care for victims with life-threatening injuries or exposures. Therefore, there exists a need for a rapidly deployable biological assay for radiation exposure that can be performed in the field by individuals with little to no medical training. Saliva is an attractive biofluid for this purpose, due to the relative ease of its collection and the wide array of biomolecules it contains. To determine whether the human salivary proteome is responsive to ionizing radiation exposure, we characterized the abundances of salivary proteins in humans before and after total body irradiation. Using an assay panel targeting 90 analytes (growth factors, chemokines and cytokines), we identified proteins that were significantly radiation responsive in human saliva. The responses of three proteins (monocyte chemo-attractant protein 1, interleukin 8 and intercellular adhesion molecule 1) were confirmed using independent immunoassay platforms and then verified and further characterized in 130 saliva samples from a completely independent set of 38 patients undergoing total body irradiation. The results demonstrate the potential for detecting radiation exposure based on analysis of human saliva.

Introduction

Radiological events can be due to accident, natural disaster (e.g. Fukushima, 2011) or the use of radiological weaponry in a terror attack. After such an incident in a heavily populated area, masses of panicked people asking to be evaluated for exposure will likely overwhelm our emergency care system and consequently jeopardize effective triage and treatment of those whose lives are in imminent danger (1–5). Thus, we need a rapid diagnostic test for screening for exposure, allowing us to rapidly triage unexposed individuals from individuals for whom further assessment (e.g. detailed biodosimetry) and dose-dependent intervention may be required.

Saliva is emerging as a premier biospecimen for diagnostic testing, since spitting into a cup or licking a diagnostic test strip does not require medical staff or training and provides an

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economical alternative to phlebotomy (6–8). Given the well-documented sensitivity of the salivary glands to radiation (9) and their intimate anatomical and functional relationship with saliva, it is feasible that radiation biomarkers in saliva will emanate from the salivary gland. Additionally, proteins present in blood are also present in saliva from leakage at the gum line and this could provide another source of radiation-responsive markers in saliva (10). Indeed, in 2004, an oral-based test for HIV was approved by the U.S. Food and Drug Administration. Currently, saliva is being actively explored as a biofluid for detection of multiple disease biomarkers, including oral squamous cell carcinoma (OSCC) (11, 12), type 2 diabetes mellitus and periodontal disease (13), lung cancer (14), pancreatic cancer (15) and breast cancer (16). Recent work has also focused on the discovery of salivary biomarkers of brain injury and disease. For example, tau proteins, which are microtubule-stabilizing proteins found in neurons of the central nervous system, have been found in saliva and phosphorylated tau/total tau protein ratios are significantly increased in patients with Alzheimer's disease (17). Two important potential biomarkers of Parkinson's disease, α -synuclein (α -Syn) and DJ-1, have also been detected in saliva (18).

There are four main salivary glands that contribute to whole saliva. The submandibular gland contributes 65% of proteins in whole saliva, while the parotid, sublingual and various minor glands contribute 23%, 4% and 8%, respectively (19). Saliva can be collected using oral stimulation by chewing on paraffin wax or gum (stimulated saliva) or it can be collected without stimulation by draining, spitting or suction. Unstimulated saliva collection remains the most commonly used method (20, 21). Recent studies have identified 2,290 proteins in whole saliva (22).

The purpose of this study was to determine the feasibility of detecting radiation exposure based on protein expression in human saliva. A 90-analyte protein assay panel was used to identify proteins that demonstrated radiation responsiveness in saliva samples collected from patients before and after total body irradiation (TBI). Three radiation-responsive proteins [monocyte chemoattractant protein 1 (MCP-1), interleukin 8 (IL-8) and intercellular adhesion molecule 1 (ICAM-1)] were verified and further characterized using independent immunoassays. In total, 147 saliva samples from 45 cancer patients undergoing TBI were analyzed. The results demonstrate the potential for detecting radiation exposure based on analysis of human saliva.

Methods

Saliva Sample Collection and Processing

All biospecimen collections for these studies were approved by the Fred Hutchinson Cancer Research Center's Institutional Review Board (Protocol 2322/IRB 6930). Unstimulated saliva samples were collected as follows: Donors rinsed their mouths with 50 mL of water prior to saliva collection and then expectorated into a 50 mL conical centrifuge tube until 5–10 mL of saliva was collected (or for no longer than 30 min). The samples were placed on ice prior to processing. Cells and large debris were removed from the samples by centrifugation (400g, 8 min at 4°C). Nonsoluble components were removed by an additional centrifugation step (20,000g, 30 min at 4°C), and the supernatants were transferred to cryotubes and stored at either –80°C or in liquid nitrogen. The protein concentration of each

sample was determined using a Micro BCA Protein Assay Kit with bovine serum albumin (BSA) as reference standard (ThermoFisher Scientific, Waltham, MA.).

Protein Assays

Discovery phase screen—An initial “discovery phase screen” for radiation-responsive proteins in human saliva was conducted using the Luminex microbead platform to run the HumanMAP v1.6® assay panel (Myriad RBM; Austin, TX), consisting of 90 analytes including growth factors, chemokines and cytokines.

Confirmation studies of IL-8 and MCP-1—To confirm the results obtained using the HumanMAP v1.6® assay panel, we used independent assays. Specifically, a sandwich immunoassay used for Human IL-8 quantification (ThermoFisher Scientific, catalog nos. M801 and M802B) was run on the Luminex platform in the Cytokine Analysis Laboratory at the Fred Hutchinson Cancer Research Center (Seattle, WA). The immunoassay used here for confirmation studies was different than the immunoassay system used in the discovery phase where the antibodies came from BD Biosciences, catalog nos. 554716 and 554718. Analyte concentration was determined by a reference standard curve (WHO/NIBSC International Standard Proteins) prepared with each assay. The sandwich immunoassay used for confirmation of Human MCP-1 was from R&D Systems® (Quantikine ELISA for Human CCL2/MCP-1, catalog no. SCP00). This is a different immunoassay system than the assay used in the discovery phase where the antibodies came from BD Biosciences, catalog no. 554664. Saliva samples from the discovery phase screen were tested again at two dilutions and measured in duplicate for each assay. Results from the lowest sample dilution that fell within the standard curve range are reported. The MCP-1 assay standard curve range is 31.2–2,000 pg/mL with a limit of detection (LOD) of 1.7 pg/mL and a limit of quantitation (LOQ) of 31.2 pg/mL. The IL-8 assay standard curve range is 0.4–800 pg/mL with an LOD of 0.4 pg/mL and an LOQ of 2.0 pg/mL.

Verification studies of IL-8 and MCP-1—Sandwich immunoassays were obtained for Human IL-8 (ThermoFisher Scientific, catalog nos. M801 and M802B) and MCP-1 (BD Biosciences, catalog nos. 555055 and 554664) and run on the Luminex platform in the Cytokine Analysis Laboratory at the Fred Hutchinson Cancer Research Center. Analyte concentration was determined by a reference standard curve (WHO/NIBSC International Standard Proteins) prepared with each assay. Each verification patient saliva sample was tested on three different days at two dilutions and measured in duplicate for each assay. Results from the lowest sample dilution that fell within the standard curve range are reported. The MCP-1 assay standard curve range is 1.5–4,000 pg/mL with an LOD of 1.9 pg/mL and an LOQ of 9.6 pg/mL. The IL-8 assay standard curve range is 0.4–800 pg/mL with an LOD of 0.4 pg/mL and an LOQ of 2.0 pg/mL.

Confirmation and verification studies of ICAM-1/CD54—Human ICAM-1 was quantified using a sandwich ELISA kit from R&D Systems (catalog no. DY720). This is different than the immunoassay system used in the discovery phase where the antibodies came from ThermoFisher (catalog no. MS-114-PABX) and R&D Systems (catalog no. BBA4). The general ELISA protocol supplied with the R&D Systems kit was followed

except for the following: plates were blocked with phosphate buffered saline (PBS), 10% SuperBlock (ThermoFisher Scientific) and 0.1% Tween-20; incubation of samples and standards was 1 h; incubation of detection antibody was 1 h; and incubation of Streptavidin-HRP was 20 min. All incubations were done at room temperature on a plate shaker. After addition of 3,3',5,5'-tetramethylbenzidine (TMB) substrate (Sigma) and color development, 0.4N hydrochloric acid (HCl) was added and absorbance was measured at 450 nm. Each saliva sample was diluted 1:2 and 1:10 in reagent diluent (1% BSA, PBS) and tested in triplicate on each ELISA plate. Results from the lowest sample dilution that fell within the standard curve range are reported. The ICAM-1 standard curve range was from 31.3–2,000 pg/mL with an LOD of 5 pg/mL and an LOQ of 20 pg/mL.

Data Analysis

Receiver operating characteristic (ROC) curves were plotted using ROCR package (version 1.0–5). The area under the curve (AUC) was derived by numerical integration of the ROC curve using ROCR package (version 1.0–5). *P* values were calculated based on the Wilcoxon test and *P* = 0.05 was used as cutoff for significance.

Results

Collection of Human Saliva Samples

Saliva samples were collected per a standard operating protocol (see the Methods section) from 45 cancer patients undergoing TBI (30 males and 15 females) in preparation for autologous or allogeneic stem cell transplant at the University of Washington Medical Center or Seattle Cancer Care Alliance. Patients were included in the study if they were receiving TBI, had received no additional radiation or chemotherapy within two weeks of the start of their TBI and were able to provide informed consent. Patient demographics, as well as clinical information, are provided in Table 1 and Supplementary Table S1 (<http://dx.doi.org/10.1667/RR13586.1.S1>), respectively.

The 45 patients in this study represented two classes of TBI exposure (Supplementary Table S1; <http://dx.doi.org/10.1667/RR13586.1.S1>). Thirty-six patients received multiple fractions of TBI (1.5, 1.65 or 2 Gy each) (Fig. 1A) with two fractions delivered per day (6–8 h apart), over several consecutive days. Nine patients received a single dose of TBI (2 Gy) (Fig. 1B) with no additional radiation treatment before the final sample collection. Pre-TBI saliva samples were collected within 3 days of the beginning of radiation treatment. Post-TBI samples were collected approximately 2 h (range 0.2–2.0 h) and again at approximately 4 h (range 2.1–8 h) after TBI and then again approximately 24 h later. The distribution of samples throughout these time ranges is plotted in Supplementary Fig. S1 (panel A, 2 h; panel B, 4 h; and panel C, 24 h) (<http://dx.doi.org/10.1667/RR13586.1.S2>). Because the patients who received single-dose TBI provided a unique opportunity to measure the duration of radiation response after a single exposure, additional samples were collected at approximately 48, 72, 120 and 168 h post-TBI. The exact timing of the collections relative to TBI was variable among the patients, depending on their availability and clinic workflows. The exact doses and times of collections are reported on a per-patient basis in Supplementary Table S1 (<http://dx.doi.org/10.1667/RR13586.1.S1>).

The patients were randomly divided into a discovery and verification set (Supplementary Table S1; <http://dx.doi.org/10.1667/RR13586.1.S1>). The discovery sample set consisted of 17 saliva samples obtained from 6 TBI patients. The verification sample set consisted of 130 saliva samples obtained from 38 TBI patients.

Discovery Phase Screen for Radiation-Responsive Proteins in Human Saliva

To determine whether the human salivary proteome is responsive to radiation, the 17 saliva samples from the discovery sample set were analyzed using the HumanMAP v1.6® multiplex immunoassay (Myriad RBM) that quantifies 90 proteins, including growth factors, chemokines and cytokines. Based on a review of the literature, we determined that 28 of the 90 proteins in the panel had been previously detected in saliva (23–28). Overall, 50% of the analyzed proteins were detected at or above the lower limit of detection of the immunoassay in these saliva samples. See Supplementary Table S2 (<http://dx.doi.org/10.1667/RR13586.1.S1>) for list of discovery phase screen protein targets, samples tested and results. Four proteins demonstrated potential radiation responsiveness based on the following criteria: (i) mean fold induction >2 at 24 h; (ii) mean analyte concentration at 24 h > mean concentration +2 standard deviation of the pre-TBI samples; and (iii) $P < 0.05$. Due to limited sample volumes, the three candidate markers with the largest mean fold induction (MCP-1, IL-8 and ICAM-1) were selected for follow-up confirmation and verification studies using independent assays and samples.

Confirmation of Discovery Phase Screen for Radiation-Responsive Proteins in Human Saliva

Next, the radiation responsiveness of these three protein targets was confirmed in the discovery cohort using different immunoassays from the assays used in the initial discovery phase screen. Figure 2 shows the results from the comparison of these three proteins in the same saliva samples using two different immunoassay systems. The trends between the two assay systems were similar for all sample types pre- and post-TBI, confirming results from the discovery phase screen. Thus, these three proteins were carried through to verification testing in a larger, independent sample set.

Verification Phase Testing of MCP-1, IL-8 and ICAM-1 for Radiation Responsiveness in Human Saliva

The radiation responsiveness of the MCP-1, IL-8 and ICAM-1 proteins was further assessed in the verification sample set, which consisted of completely different TBI patients than those in the discovery set (Supplementary Table S1; <http://dx.doi.org/10.1667/RR13586.1.S1>). Additionally, the immunoassays used to quantify these 3 proteins in the verification sample set were different from those used in the discovery screen (see the Methods section). In the verification study, we tested 130 saliva samples from 38 patients undergoing TBI. These samples represent 82 saliva samples from 29 multi-fraction TBI patients and 48 saliva samples from 9 single-dose TBI patients.

The median MCP-1 concentration was 500 pg/mL in pre-TBI samples from single-dose TBI patient saliva (8 samples). The MCP-1 levels increased after 2 Gy irradiation to approximately 1,500 pg/mL at 2 h and up to approximately 18,000 pg/mL at 4 h after TBI.

These levels remained elevated for up to 48 h after radiation treatment (Fig. 3A). MCP-1 levels in saliva from patients receiving multiple fractions of TBI also increased after irradiation. Pre-TBI patient saliva had a median MCP-1 concentration of 200 pg/mL (28 samples). After TBI, the MCP-1 levels in these patients increased to approximately 1,200 pg/mL at 2 h, approximately 7,600 pg/mL at 4 h and approximately 5,600 pg/mL at 24 h (Fig. 3B). These represent post-TBI increases of 6-, 38- and 28-fold after 2, 4 and 24 h, respectively. All data from MCP-1 verification testing are available in Supplementary Table S3 (<http://dx.doi.org/10.1667/RR13586.1.S1>). ROC curves were generated for samples from patients receiving multiple fractions of TBI to determine whether the difference between irradiated and nonirradiated MCP-1 concentrations were significant at different time points (Fig. 3C). The area under the curve for the ROC curves was 0.74 after 2 h ($P = 0.004$), 0.93 after 4 h ($P < 0.0001$) and 0.89 at 24 h ($P < 0.0001$) after the initial dose of TBI. The results were comparable whether or not MCP-1 levels were normalized to total protein concentration (Supplementary Tables S4 and S5; <http://dx.doi.org/10.1667/RR13586.1.S1>). Additionally, ROC curves were generated from combined single-dose TBI and multiple fraction TBI at 2 and 4 h, since all of these samples were collected after only one radiation dose. The AUC values of the combined ROC curves were comparable to the values using only data from multiple fraction patient samples (Supplementary Fig. S2; <http://dx.doi.org/10.1667/RR13586.1.S2>).

The median IL-8 concentration was 130 pg/mL in pre-TBI samples from single-dose TBI patient saliva (8 samples) and increased after 2 Gy irradiation to approximately 680 pg/mL after 2 h and to approximately 5,000 pg/mL after 4 h (Fig. 4A). IL-8 levels remained elevated for 48 h after a single fraction of 2 Gy TBI. IL-8 levels in saliva from patients receiving multiple fractions of TBI also increased after irradiation. The pre-TBI patient saliva had a median IL-8 concentration of 300 pg/mL (28 samples). After TBI treatment, the IL-8 levels in these patients increased to approximately 500 pg/mL at 2 h, 3,500 pg/mL at 4 h and 2,000 pg/mL at 24 h (Fig. 4B). These represent post-TBI increases of 1.7-, 12- and 7-fold after 2, 4 and 24 h, respectively. All data from IL-8 verification testing are available in Supplementary Table S6 (<http://dx.doi.org/10.1667/RR13586.1.S1>). ROC curves were generated for data from patients receiving multiple fractions of TBI to determine whether the difference between irradiated and nonirradiated IL-8 concentrations were significant (Fig. 4C). The AUC for the ROC curves was 0.55 at 2 h ($P = 0.3$), 0.9 at 4 h ($P < 0.0001$) and 0.79 at 24 h ($P = 0.0004$) after TBI. The results were comparable whether or not IL-8 levels were normalized to total protein concentration (Supplementary Tables S4 and S5; <http://dx.doi.org/10.1667/RR13586.1.S1>). Additionally, ROC curves were generated from combined single-dose TBI and multiple fraction TBI at 2 h and 4 h, since all of these samples were collected after only one radiation dose. The AUC values of the combined ROC curves were comparable to the values using only data from multiple fraction patient samples (Supplementary Fig. S3; <http://dx.doi.org/10.1667/RR13586.1.S2>).

The median ICAM-1 concentration was 1,000 pg/mL in pre-TBI samples from single-dose TBI patient saliva (8 samples) and increased after 2 Gy irradiation up to approximately 3,500 pg/mL after 4 h and peaked at approximately 6,400 pg/mL 48 h after TBI (Fig. 5A). In saliva from patients receiving multiple fractions of TBI, ICAM-1 concentrations also

increased after irradiation. Pre-TBI patient saliva had a median ICAM-1 concentration of 700 pg/mL (28 samples). After TBI treatment, the levels in these patients increased to approximately 3,000 pg/mL after 4 h and 7,700 pg/mL after 24 h (Fig. 5B). These represent post-TBI median increases of 4- and 11-fold after 4 and 24 h, respectively. All data from ICAM-1 verification testing are available in Supplementary Table S7 (<http://dx.doi.org/10.1667/RR13586.1.S1>). ROC curves were generated for samples from patients receiving multiple fractions of TBI to determine whether the difference between irradiated and nonirradiated ICAM-1 concentrations were significant (Fig. 5C). The area under the curve for the ROC curves was 0.5 at 2 h ($P=0.49$), 0.85 at 4 h ($P < 0.0001$) and 0.96 at 24 h ($P < 0.0001$) after TBI. The results were comparable whether or not ICAM-1 levels were normalized to total protein concentration (Supplementary Tables S4 and S5; <http://dx.doi.org/10.1667/RR13586.1.S1>). Additionally, ROC curves were generated from combined single-dose TBI and multiple fraction TBI at 2 and 4 h, since all of these samples were collected after only one radiation dose. The AUC values of the combined ROC curves were comparable to the values using only data from multiple fraction patient samples (Supplementary Fig. S4; <http://dx.doi.org/10.1667/RR13586.1.S2>).

Discussion

Our findings demonstrate the feasibility of identifying individuals exposed to radiation by direct analysis of human saliva. To be useful for population screening during a radiological incident, radiation biomarkers need to be sensitive enough to detect clinically relevant exposures (at least down to 2 Gy), specific to radiation, elevated rapidly (within an hour) for initial population screening and triage and remain elevated long enough for an emergency response to be mounted (3–5 days). The ideal radiation biomarkers would also be predictive of patient outcome (including both acute and long-term effects), facilitating dose-dependent interventions.

In this study, we identified MCP-1, IL-8 and ICAM-1 as being radiation responsive in human saliva. All three proteins are mediators of the inflammatory response and have been previously identified in saliva (28) but not as salivary biomarkers of radiation injury. MCP-1 and IL-8 belong to a class of cytokines known as chemokines which are small signaling proteins that cause migration of specific cell types to the site of injury (29). MCP-1 attracts monocytes and basophils, and IL-8 attracts neutrophils; both proteins are produced by macrophage, endothelial cells, dendritic cells and other cell types in response to the proinflammatory cytokines, TNF α and IL-1 β (29, 30). Cytokines and chemokines are known players in the immediate and latent response to radiation throughout the body (31, 32). Studies have shown an increase in MCP-1 messenger RNA and protein expression after radiation in rat brains (cortex and hippocampus) (33) and rat lung tissue for up to 26 weeks post-exposure (34). IL-8 is a member of the CXC chemokine family and plays a causative role in the acute inflammatory response by activating neutrophils (35, 36). IL-8 and MCP-1 have also been shown to play a role in the inflammatory response after traumatic brain injury (37). ICAM-1, also known as CD54, is an adhesion molecule and is expressed on cells of the immune system and vascular endothelium, where it aids in the transmigration of leukocytes into injured tissues (38). Prior studies have demonstrated a late and persistent upregulation of ICAM-1 after irradiation in human endothelial cells (39). ICAM-1 has also

been shown as a requirement for radiation-induced migration of inflammatory cells in lung endothelial tissue (40) and ICAM-1 gene expression is increased after irradiation as low as 2 Gy (41).

Consistent with our findings, recent reports have shown the role of the inflammatory immune response in radiation-induced tissue injury, in both acute and late effects (32, 42). Given the general role of MCP-1, IL-8 and ICAM-1 in the inflammatory immune response, their salivary concentrations may conceivably be elevated by injuries or disease states not associated with radiation injury. For example, IL-8 has been shown to play a role in periodontal disease (36, 43). Thus, a large-scale study will be required to characterize the reference ranges of these proteins normally encountered in the nonirradiated population, to assess whether cut points associated with high specificity for radiation can be determined. Additionally, salivary analyte concentrations vary from day-to-day and throughout the day within an individual (44). Thus, establishing reference ranges will require not only sampling across the population, but also longitudinally over time within an individual. Although our study was not powered to detect such effects, other factors could have an effect on the radiation responsiveness of these proteins, such as smoking status, gender and age.

It may well prove difficult to identify biomarkers truly specific for radiation exposure in human saliva, as most markers might be associated with tissue leakage or the inflammatory response and thus also affected by non-radiological insults. If this is the case, one strategy might be to assemble a panel of radiation markers that together may provide higher specificity than inflammatory markers alone. Also, given the enormity of the problem associated with screening panicked masses after a radiological event in an urban setting, a biomarker panel with high sensitivity might still be of value as an initial triage tool, even if not completely specific. In this situation, a secondary screen of individuals testing positive would be required to distinguish between true and false positives. Because existing secondary screens are time consuming and require medical personnel, a biomarker(s) enabling initial triage, even if imperfect, could greatly facilitate efficient use of resources under emergency, disaster-management conditions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Patient treatments and saliva sample collection schedules. Panel A: Multiple fraction TBI patient sample collection schedule. Patients received two fractions of radiation per day over a three-day period. The dose per fraction varied by patient from 1.5–2.0 Gy per fraction. Saliva samples were collected before (Pre) and at approximately 2, 4 and 24 h after the first fraction of radiation. Note that the 2 and 4 h samples were collected between the first and second fractions of TBI while the 24 h sample was collected after the third fraction of TBI. Panel B: Single dose TBI patient sample collection schedule. Patients received a single 2 Gy dose of radiation. Saliva samples were collected before (Pre) and at approximately 2, 4, 24, 48, 72, 120 and 168 h after the single dose of radiation.

Fig. 2.

The radiation responsiveness of MCP-1, IL-8 and ICAM-1 are confirmed using independent assays. Saliva levels of MCP-1 (panel A), IL-8 (panel B) and ICAM-1 (panel C) in the discovery phase saliva sample set (17 samples total). Saliva samples were collected from 6 patients before (Pre) and approximately 2–4 h after the initial fraction of radiation and again at 24 h after the initial dose. The same saliva samples were tested in 2 different immunoassay platforms: D = discovery phase testing platform (Myriad RBM) and C = confirmation phase testing platform (FHCRC). Plots display the Log_2 concentration (pg/mL) for each sample in both assay platforms. The median concentration is indicated by the horizontal bars while the boxes represent middle quartiles and dashed lines represent the 95% confidence interval.

Fig. 3.

The radiation responsiveness of MCP-1 is verified in an independent set of patients. Each sample was measured in triplicate and the median $\log_2$ concentration (pg/mL) is plotted for each sample. The median concentration for all samples at a given time point is indicated by the horizontal bars, while the boxes represent the middle quartiles and dashed lines the 95% confidence interval. Panel A: Levels of MCP-1 measured by immunoassay in patients receiving a single dose of radiation. Saliva was collected from 9 patients before (Pre, $n = 8$) and after a single 2 Gy fraction of TBI at 2 h ($n = 5$), 4 h ($n = 6$), 24 h ($n = 6$), 48 h ($n = 7$), 72 h ($n = 4$), 120 h ($n = 6$) and 148 h ($n = 6$ each). Panel B: Levels of MCP-1 measured by immunoassay in patients receiving multiple fractions of TBI. Saliva samples were collected from 29 patients before (Pre, $n = 28$) the initial fraction of TBI, as well as 2 h ($n = 15$), 4 h ($n = 21$) and 24 h ($n = 18$) after the initial fraction of TBI. Panel C: ROC analysis of MCP-1 levels in saliva samples from patients receiving multiple fractions of TBI (same samples as listed for panel B). The curves are drawn for the 2, 4 and 24 h samples as indicated. The area under the curve (AUC) is listed along with the associated P values for each time point.

Fig. 4.

The radiation responsiveness of IL-8 is verified in an independent set of patients. Each sample was measured in triplicate and the median $\log_2$ concentration (pg/mL) is plotted for each sample. The median concentration for all samples at a given time point is indicated by the horizontal bars, while the boxes represent the middle quartiles and the dashed lines the 95% confidence interval. Panel A: Levels of IL-8 measured by immunoassay in patients receiving a single dose of radiation. Saliva was collected from 9 patients before (Pre, $n = 8$) and after a single 2 Gy fraction of TBI at 2 h ($n = 5$), 4 h ($n = 6$), 24 h ($n = 6$), 48 h ($n = 7$), 72 h ($n = 4$), 120 h ($n = 6$) and 148 h ($n = 6$ each). Panel B: Levels of IL-8 measured by immunoassay in patients receiving multiple fractions of TBI. Saliva samples were collected from 29 patients before (Pre, $n = 28$), 2 h ($n = 15$), 4 h ($n = 21$) and 24 h ($n = 18$) after the initial fraction of TBI. Panel C: ROC analysis of IL-8 levels in saliva samples from patients receiving multiple fractions of TBI (same samples as listed for panel B). The curves are drawn for the 2, 4 and 24 h samples as indicated. The area under the curve (AUC) is listed along with the associated P values for each time point.

Fig. 5.

The radiation responsiveness of ICAM-1 is verified in an independent set of patients. Each sample was measured in triplicate and the median Log₂ concentration (pg/mL) is plotted for each sample. The median concentration for all samples at a given time point is indicated by the horizontal bars, while the boxes represent the middle quartiles and dashed lines the 95% confidence interval. Panel A: Levels of ICAM-1 measured by immunoassay in patients receiving a single dose of radiation. Saliva was collected from 9 patients before (Pre, n = 8) and after a single 2 Gy fraction of TBI at 2 h (n=5), 4 h (n=6), 24 h (n=6), 48 h (n=7), 72 h (n=4), 120 h (n=6) and 148 h (n = 6 each). Panel B: Levels of ICAM-1 measured by immunoassay in patients receiving multiple fractions of TBI. Saliva samples were collected from 29 patients before (Pre, n = 28), 2 h (n = 15), 4 h (n = 21) and 24 h (n = 18) after the initial fraction of TBI. Panel C: ROC analysis of ICAM-1 levels in saliva samples from patients receiving multiple fractions of TBI (same samples as listed for panel B). The curves are drawn for the 2, 4 and 24 h samples as indicated. The area under the curve (AUC) is listed along with the associated *P* values for each time point.

Table 1
Donor Demographic Summary

TBI patients (n = 45)	
Median age (years)	43
Age range	9–58
Gender	
Number of males	30
Number of females	15
Ethnicity	
Caucasian	39
Others	6
Smoking history*	
Never	29
Past	8
Current	8

* Smoking history categories: Past = nonsmoker for 6 months or longer; Current = history of smoking within the last 6 months.