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Clinical Investigation

Serum Proteome Signature of Radiation Response: Upregulation of Inflammation-Related Factors and Downregulation of Apolipoproteins and Coagulation Factors in Cancer Patients Treated With Radiation Therapy—A Pilot Study

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Summary

RT-induced effects were analyzed systemically and quantitatively at the level of complete human serum proteome for the first time. Patients being treated for head and neck squamous cell carcinoma and prostate cancer were enrolled. Signature of response to radiation was identified, which included upregulation of inflammation factors in the acute phase and downregulation of plasma

Purpose: Ionizing radiation affects the proteome of irradiated cells and tissue, yet data concerning changes induced during radiation therapy (RT) in human blood are fragmentary and inconclusive. We aimed to identify features of serum proteome and associated processes involved in response to partial body irradiation during cancer treatment.

Methods and Materials: Twenty patients with head and neck squamous cell cancer (HNSCC) and 20 patients with prostate cancer received definitive intensity modulated RT. Blood samples were collected before RT, just after RT, and 1 month after the end of RT. Complete serum proteome was analyzed in individual samples, using a shotgun liquid chromatography-tandem mass spectrometry approach which allowed identification of approximately 450 proteins. Approximately 100 unique proteins were quantified in all samples after exclusion of immunoglobulins, and statistical significance of differences among consecutive samples was assessed. Processes associated with quantified proteins and their functional interactions were predicted using gene ontology tools.

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lipoproteins and coagulation factors. Moreover, changes observed were associated with type of acute radiation toxicity rather than with a dose-volume effect.

Results: RT-induced changes were marked in the HNSCC patient group: 22 upregulated and 33 downregulated proteins were detected in post-RT sera. Most of the changes reversed during follow-up, yet levels of some proteins remained affected 1 month after the end of RT. RT-upregulated proteins were associated with acute phase, inflammatory response, and complement activation. RT-downregulated proteins were associated with transport and metabolism of lipids (plasma apolipoproteins) and blood coagulation. RT-induced changes were much weaker in prostate cancer patients, which corresponded to differences in acute radiation toxicity observed in both groups. Nevertheless, general patterns of RT-induced sera proteome changes were similar in both of the groups of cancer patients.

Conclusions: In this pilot study, we proposed to identify a molecular signature of radiation response, based on specific features of serum proteome. The signature included upregulation of factors involved in acute or inflammatory response but also downregulation of plasma apolipoproteins and factors involved in blood coagulation.

Introduction

Molecular signatures of human serum proteome could potentially be used for detection and/or classification of human diseases, including cancer (1-3). Several components of these signatures, especially those characteristic of advanced cancer, were identified as proteins (or their fragments) involved in systemic response of the patient's body to the disease (eg, inflammation). Furthermore, profiling of serum plasma proteome of patients undergoing anticancer therapy revealed features apparently associated with general toxicity of the treatment to the patient's organism (4-6). There are several reports documenting effects of ionizing radiation (eg, partial body irradiation received during cancer patients' radiation therapy [RT]). Molecular changes observed in sera from patients undergoing RT apparently reflected pleiotropic effects of the treatment, which could be associated with either regression of tumor and/or toxicity in normal tissues. It was shown that affects to proteins in sera of humans exposed to radiation are associated with many different molecular processes, yet factors involved in immunity and inflammation response appeared to be the most important (7-13). However, systemic and quantitative determination of a human serum proteome signature of response to radiation presently remains missing.

RT is an effective treatment of head and neck squamous cell cancer (HNSCC), yet these patients' advanced cases require intensive treatment schemes, which are associated with increased risk of toxicity (14, 15). Acute mucosal reaction (AMR) in HNSCC patients is caused by damage of epithelial cells in oral mucosa, a large volume of which is frequently irradiated with relatively high doses (15, 16). Recent treatment of HNSCC is frequently provided using intensity modulated RT (IMRT), where a high dose conforms better to the tumor shape and allows reduced dose to adjacent critical organs. However, a potential drawback of IMRT is the exposure of a large volume of normal tissues to low or medium doses, which could further affect whole-body response to treatment. Radiation-induced changes in blood proteome have been studied in patients undergoing IMRT for treatment of HNSCC, and marked changes have been detected in the low-molecular-weight fraction of serum proteome (so-called endogenous peptidome). Importantly, an apparent correlation between serum peptidome features and volume of irradiated normal tissues or intensity of AMR has been established (12). Hence, RT-related changes observed in serum proteome of HNSCC patients most likely reflects acute toxicity induced in normal tissues. However, actual identity and function of serum proteome components affected by radiation in HNSCC patients has not been established.

In this study we aimed to define the serum proteome signature of radiation response and to establish the role of molecular and cellular processes involved in response to partial body irradiation during cancer treatment. Radiation-related effects were assessed at the level of complete serum proteome of HNSCC patients, and affected proteins were mapped in the network of molecular pathways. Moreover, RT-related features of serum proteome of prostate cancer patients who received lower radiation toxicity were analyzed for comparison.

Methods and Materials

Characteristics of patient groups

Twenty patients (Caucasian; 45-76 years of age; median, 60 years of age; 16 men) with HNSCC were enrolled in the study. Cancer was located in either the oral cavity (2 patients), oropharynx (5 patients), hypopharynx (3 patients), or larynx (10 patients); primary tumors were assessed T2 (45%) and T3 (55%) stage; 40% of patients had stage N0. Additionally, a group of 20 men