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Ionizing radiation-induced altered microRNA expression as biomarkers for assessing acute radiation injury

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1. Introduction

Accidental or deliberate radiation exposure is a grave threat to humans and a major public health concern that requires prompt attention for accurate diagnosis and appropriate clinical planning to ensure a better outcome in exposed victims. In the event of a nuclear or improvised nuclear device incident, deliberate attack by terrorists, or a nuclear power plant accident, thousands of people may be exposed to high doses of ionizing radiation. The effects may vary greatly. Some victims may be asymptomatic while others may exhibit mild to servere symptoms, resulting in death in some cases. In the case of human acute radiation syndrome (ARS), following exposure to acute radiation doses of >2 Gy, the major sub-syndromes include hematopoietic (2 – 6 Gy), gastrointestinal (6 – 10 Gy), and neurovascular (>10 Gy).

It is a challenging task for medical responders to triage ionizing radiation-exposed victims into definable, treatment-susceptible groups in a mass casualty scenario. For example, minimally-exposed (<2 Gy) individuals may not require immediate care. Victims with exposure to moderate (2 – 6 Gy) or high doses (6 – 10 Gy) of radiation can most likely benefit from timely treatment. However, those who have received supralethal doses (>10 Gy)

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Declaration of Interest

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of radiation, and cannot be treated to survive, need only palliative care. Identifying these subcategories is essential to conserve the scarce resources anticipated to be available during any mass casualty scenario. The problem is that the currently prevalent strategies to assess the status of individuals exposed to various doses of radiation are for the most part based on signs and symptoms developing over time or biological dosimetry. Examples of such signs and symptoms include determination of the time of exposure to time of onset/severity of nausea/vomiting, cytogenetic analysis such as the dicentric assay, and kinetics of lymphocyte depletion. However, cytogenetic analysis for dicentrics and lymphocyte kinetic assays are time-consuming, labor-intensive, and difficult to execute in a mass casualty scenario. Therefore, it is reasonable to develop non-invasive biomarkers for radiation exposure based on molecular changes such as DNA, RNA, protein, and metabolites [1,2].

Retrospectively, candidates for such non-invasive radiation exposure biomarkers have included C-reactive protein, cytokines, chemokines, growth factors, amylase, and other proteins [1]. However, there are concerns with a protein-based approach, including the intrinsic complexity of proteins (encompassing post-translational modification), the difficulty in developing a detection assay with high affinity, and the frequent low abundance of protein of interest. Adding to these difficulties, protein biomarkers can be affected by various physiological conditions such as infection and inflammation. Turning to nucleic acids, the messenger RNA (mRNA) expression has been used to predict radiation exposure dose. mRNA is inherently unstable, making them especially difficult to use as dependable biomarkers for radiation injuries in the field. However, one benefit of using mRNA as a radiation exposure biomarker is the presence of well-defined housekeeping genes. DNA double-strand break sites and γ-H2AX are being investigated to serve as biomarkers for assessing the dose of radiation exposure. γ-H2AX applicability is restricted to the first minutes or half an hour after exposure due to fast and efficient DNA repair. Additionally, plasma B1 DNA appeared to be a likely biomarker for radiation exposure. Similarly, plasma DNA levels can significantly vary as a result of many comorbid conditions.

MicroRNAs (miRNAs), a class of small non-coding RNAs, may prove to be radiation exposure biomarkers that bypass many of these difficulties. In general, non-coding RNAs include multiple classes of RNA transcripts that are not transcribed into proteins. Nonetheless, they have been found to serve important roles in regulating the rate of gene transcription, stability of transcripts, and translation of proteins [3]. Within three decades, the study of miRNA has grown exponentially in eukaryotes, and it has now been well-established that these small RNAs are important regulators of gene expression and several other biological processes. The measurement of miRNAs depends on a simple technology, and miRNA have the potential to be useful diagnostic, prognostic, and predictive biomarkers. miRNAs regulate gene expression by complementary base pairing with the 3′-untranslated region of target mRNA. The partial duplex between a miRNA and its target mRNA is then a target for nucleases and therefore rapid degradation. In the absence of nuclease attack, the duplex prevents translation by blocking progression of the target mRNA through the ribosome. The result is a loss of the target protein. Recent studies have suggested that the presence of miRNAs in biofluids such as blood or urine might be complementary to already examined and partly accepted biomarker on the protein or mRNA level [4]. There are several advantages of using circulating miRNAs as radiation exposure

biomarkers. Such advantages include the fact that miRNA expression usually changes as a result of disease and organ damage. In addition, miRNAs are tissue specific, stable at harsh environmental conditions, evolutionarily conserved across species, and can be measured easily in efficient, high-throughput analyses. miRNAs are stable, not only in serum and plasma, but also in tissues fixed in formalin. Several studies have identified circulating miRNAs found in apoptotic bodies, exosomes, high density lipoprotein, and RNA binding proteins as a form of a cell-to-cell communication channel.

Recent studies suggest that serum miRNA can serve as functional dosimeters for early assessment of radiation injury. In terms of speed of initiating therapy, early detection of biomarkers to assess radiation exposure is crucial for timely intervention with countermeasures. Many treatment options such as Neupogen, Neulasta, cytokines, and antibiotics need to be initiated prior to the onset of symptoms for the optimal outcome. Consequently, the delayed administration of most countermeasures is not effective, and therefore serves to emphasize the need for early detection of radiation exposure biomarkers. The stability of biofluid miRNA under severe physical conditions and their reproducibility make miRNAs attractive candidates as noninvasive radiation exposure biomarkers. This will be critical for the timely use of radiation countermeasures to mitigate impact of radiation exposure. There are several studies using rodent and nonhuman primate (NHP) models suggesting that miRNAs can predict both the extent of radiation injury and outcome for survival/mortality, and they may serve as an ideal biomarker choice [1,5–8].

2. Studies with miRNAs in animal models of ARS

Effects of radiation exposure on miRNA expression have been conducted using several animal species. In the case of ARS, miRNA analysis has been performed using murine and NHP models. The mouse is one of the most characterized species and the most frequently used animal model in terms of studying radiation injury. During the last few years, several studies identified miRNAs as biomarkers for radiation injury and radiation countermeasure efficacy using various strains (CD2F1, C57BL/6J, C57BL/6, and CBA/J) of mice. Though large numbers of miRNAs have been demonstrated to be altered in response to irradiation, not all studies have shown modulation of the same miRNAs in response to radiation exposure [9–13]. Still, the majority of studies have demonstrated the downregulation of miR-150 and upregulation of both miR-30 and miR126. miR-150 was downregulated by ^{60}Co γ-radiation exposure as well as high LET and high energy particle exposure (^{56}Fe, iron-56) [11]. In addition to total-body irradiation, miR-150 downregulation has also been shown in lung and blood of whole thorax irradiated (15 Gy at dose rate of 1.43 Gy/min) female WAG/RijCmcr rats [8], suggesting potential of using miRNAs for partial-body exposure, and the influence on miRNA expression in organ and biofluids under such situation.

The use of NHP models to identify miRNAs as biomarkers for radiation injury or countermeasure efficacy has special relevance since the NHP is closely related to humans, sharing >95% DNA sequence homology and possessing a high level of similarity in terms of response to various physiological pathways and cell receptors, more so than any other animal model. The majority of studies have been conducted using the rhesus macaque,

although there is also a report with baboons exposed to radiation. A recently conducted study using rhesus macaques identified a signature of seven significantly altered miRNAs (miR-150–5p, miR-215– 5p, miR-30a-5p, miR-126–5p, miR-133a-3p, miR-133b-3p, and miR-375–3p) 24 h following exposure to ionizing radiation [5]. Differences in the expression of three miRNAs (miR-133b, miR-215, and miR-375) were able to distinguish irradiated versus un-irradiated NHPs with complete accuracy. In this elegant study, two miRNAs (miR-30a and miR-126) were able to predict radiation-induced mortality in NHPs. Another study using rhesus macaques also demonstrated the upregulation of miR-126–3p and downregulation of miR-150–5p [6]. In a study using the baboon model, unlike rhesus macaques, miR-342–3p was found to be most affected (10-fold sustained downregulation) at 24 and 48 h post-irradiation compared to samples collected from animals prior to irradiation [7].

miR126–3p is interesting since it was upregulated in both mice and rhesus macaques in response to ^{60}Co γ-radiation exposure. A promising radiation countermeasure under advanced development, γ-tocotrienol (GT3), was able to revert it to preirradiation levels in mice as well as in NHPs [5,14]. In fact, three miRNAs (miR-30a, miR-126, and miR-375) correlated with the radioprotective efficacy of GT3; these miRNA in GT3-treated irradiated NHPs resembled the unirradiated animals. There is no report of miRNA analysis for humans exposed to radiation doses capable of inducing ARS during any radiological accident. The samples from cancer patients are less than optimal because of several confounders.

3. Pros and cons of miRNA

Obviously, non-invasive circulatory miRNAs have potential for biomarkers, however additional research will be needed to answer issues associated with its use. The key advantages of circulatory miRNAs includes its existence in the biofluids (liquid biopsy). Generally plasma, serum, whole blood (also urine, saliva, and) and peripheral blood cells have been used to detect circulating miRNAs. Now organ specific miRNA are also analyzed. Usually RT-qPCR, microarray platforms, nanostring technique, next-generation sequencing and biosensors are capable of detecting miRNAs in biofluids. Harmonization in the techniques for detecting and validating miRNA will be an important step. The small number of miRNA signatures (identified human miRNAs are 1/10th of genes) are unique to the altered pathways in any pathological state. miRNAs are the potential diagnostic biomarker and can also serve as prognostic and treatment monitoring biomarker. Unfortunately, there are conflicting reports which questions the specificity of miRNAs for particular pathology. Sometime experimental variables in the miRNA detection triggers lack of reproducibility.

4. Conclusions

As stated above, recent studies using murine and NHP models have identified several circulating miRNA that are altered in response to radiation exposure. These studies are for the most part based on total-body irradiation. However, results of these studies are somewhat divergent, and the reasons are not immediately apparent. It could be that the divergent miRNA profiles may be due to the different profiling platforms used in various studies [15].

Next generation platforms are increasingly available and may provide the best quantitative information, which could partly help overcoming issues of normalization.

Samples collected within the first 24 h following radiation exposure are important but may not provide the ultimate solution. It may not be realistic that radiation-exposed victims report within 24 h of exposure to medical centers that offer biomarker investigation and provide radiomitigators in either a mass casualty scenario or a radiation accident at a remote site.

Future investigations should concentrate on defining the cell population from which various identified miRNAs originate and their physiological relevance. Such studies will demonstrate whether miRNAs in biofluids discriminate between radiation-associated miRNA levels which are associated with some acute effects but not causing them

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