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Biology Contribution

Accurate Gene Expression-Based Biodosimetry Using a Minimal Set of Human Gene Transcripts

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Summary

Rapid biological dosimetry will be needed after a large-scale nuclear incident. Current methods are too slow and cumbersome for field-based triage of victims. To address this need we used reverse-transcriptase real-time polymerase chain reaction, a rapid and reliable molecular method, to identify radiation-responsive gene transcripts. Our results show that only 3 to 4 different transcripts are needed for accurate dosimetry. Analyses of these transcripts may be useful in a field-portable device

Purpose: Rapid and reliable methods for conducting biological dosimetry are a necessity in the event of a large-scale nuclear event. Conventional biodosimetry methods lack the speed, portability, ease of use, and low cost required for triaging numerous victims. Here we address this need by showing that polymerase chain reaction (PCR) on a small number of gene transcripts can provide accurate and rapid dosimetry. The low cost and relative ease of PCR compared with existing dosimetry methods suggest that this approach may be useful in mass-casualty triage situations.

Methods and Materials: Human peripheral blood from 60 adult donors was acutely exposed to cobalt-60 gamma rays at doses of 0 (control) to 10 Gy. mRNA expression levels of 121 selected genes were obtained 0.5, 1, and 2 days after exposure by reverse-transcriptase real-time PCR. Optimal dosimetry at each time point was obtained by stepwise regression of dose received against individual gene transcript expression levels.

Results: Only 3 to 4 different gene transcripts, ASTN2, CDKN1A, GDF15, and ATM, are needed to explain $\geq$0.87 of the variance (R^2). Receiver-operator characteristics, a measure of sensitivity and specificity, of 0.98 for these statistical models were achieved at each time point.

Conclusions: The actual and predicted radiation doses agree very closely up to 6 Gy. Dosimetry at 8 and 10 Gy shows some effect of saturation, thereby slightly diminishing the ability to quantify higher exposures. Analyses of these gene transcripts may be advantageous for use in a field-portable device designed to assess exposures in mass casualty situations or in clinical radiation emergencies. © 2014 Elsevier Inc.

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designed to provide rapid exposure assessments in mass casualty situations.

Introduction

Rapid and reliable quantification of human exposures to ionizing radiation remains problematic. If a radiologic device is exploded in a large city, thousands of people may be exposed. Triaging victims meaningfully according to their radiation exposure levels is not possible with existing technologies. The first step in rapidly triaging numerous people is to determine which individuals received a dose above a set threshold and therefore require medical attention. Often 2 Gy of acute whole-body exposure is considered to be the dose above which medical intervention is indicated (1, 2).

Numerous approaches exist for performing biodosimetry, and many of these have recently been compared (3). Perhaps the most frequently used method has been the evaluation of structural chromosome aberrations (4-7), but these analyses can be costly and take weeks or months to conduct, especially on large numbers of subjects. However, dicentric analyses can be useful shortly after exposure, and recent studies have evaluated this endpoint for use in triage (8-10). Micronuclei hold similar promise (11, 12), as do glycophorin A (4, 13, 14) and hypoxanthine phosphoribosyltransferase (4, 15), but these assays are less accurate than cytogenetics (4). Electron paramagnetic resonance has also been used (14), and recent improvements in this method have been made (16-18). The analysis of γ-H2AX foci also appears to have promise (19). However, none of these existing methods is fully suitable for evaluations leading to triage in mass casualty situations because they are slow and expensive, require highly skilled laboratory personnel, and/or entail sending samples to a laboratory rather than having the analytic capability at the triage location.

Of paramount importance is the development of analytic methods that overcome these issues inherent in existing biodosimetry systems. Analysis of gene transcript levels in peripheral blood by reverse-transcriptase real-time polymerase chain reaction (PCR) (qPCR) provides solutions to many of these difficulties (20). Blood-based analyses provide estimates of the average exposure to the whole body, avoiding dosimetry errors that may arise from methods that are capable only of estimating exposures in specific body parts. This is important because acute hematologic and gastrointestinal radiation toxicity is most likely related to average whole-body exposure. Transcripts from different genes can be evaluated in parallel, allowing for simultaneous replicates and internal controls. PCR-based analyses are automatable, rapid, and highly accurate, and the requisite devices can be small and easily transported in an emergency. PCR can be performed on microliter volumes of blood available from a finger-prick, eliminating the need for emergency workers who are trained in phlebotomy. Finally, operation of a semiautomated qPCR device can be accomplished with less skilled laboratory personnel than are those required for many other analytic methods.

Here we describe qPCR-based analyses of irradiated human whole blood from 60 donors for the purpose of identifying a minimal set of gene transcripts capable of providing accurate dosimetry at postexposure times relevant to triage. Some transcripts evaluated showed promise for use in biodosimetry because they exhibited persistent dose responses. Other transcripts were useful as endogenous controls because they did not exhibit substantive changes with dose or time. Coupled with the comparatively low cost, rapid analysis times, and relative ease compared with other dosimetry methods, the evaluation of gene expression by qPCR provides considerable promise as a rapid and reliable method of performing radiation biodosimetry, which may be useful in mass casualty situations.

Table 1 M values and statistics for endogenous control genes

Probe	M value	% of samples yielding valid data	SD of average Ct value
MLH1-Hs00179866_m1	0.459	97.9%	1.35
GLG1-Hs00939453_m1	0.465	99.2%	1.54
DPM1-Hs00187270_m1	0.473	99.3%	1.48
GTF3A-Hs00157851_m1	0.482	99.3%	1.42
RB1-Hs01078066_m1	0.539	99.0%	1.27

Abbreviations: Ct = cycle threshold; M value = stability factor (6); SD = standard deviation.

Methods and Materials

A full description of the materials and methods, including radiation exposures and dosimetry, RNA isolation and cDNA synthesis, real-

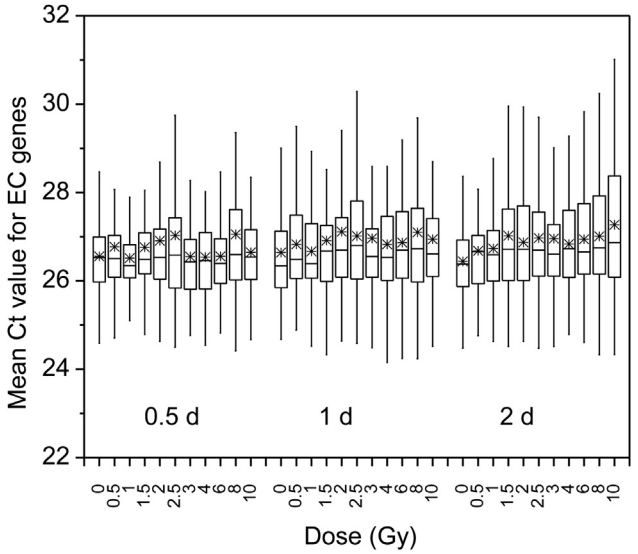

Fig. 1. Endogenous control (EC) gene cycle threshold (Ct) values by dose and time since exposure. The EC genes are DPM1, GLG1, GTF3A, MLH1, and RB1 (Table 1). These values are remarkably constant across dose and time, indicating their suitability for use as controls. The sham-irradiated and naïve data were pooled for these analyses. The top and bottom of each box indicate the 25th and 75th percentiles, vertical lines are 1.5 times the interquartile range, horizontal lines are the medians, and asterisks are the means.