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Divergent and dynamic activity of endogenous retroviruses in burn patients and their inflammatory potential

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ABSTRACT

Genes constitute ~3% of the human genome, whereas human endogenous retroviruses (HERVs) represent ~8%. We examined post-burn HERV expression in patients' blood cells, and the inflammatory potentials of the burn-associated HERVs were evaluated. Buffy coat cells, collected at various time points from 11 patients, were screened for the expression of eight HERV families, and we identified their divergent expression profiles depending on patient, HERV, and time point. The population of expressed HERV sequences was patient-specific, suggesting HERVs' inherent genomic polymorphisms and/or differential expression potentials depending on characteristics of patients and courses of injury response. Some HERVs were shared among the patients, while the others were divergent. Interestingly, one burn-associated HERV *gag* gene from a patient's genome induced IL-6, IL-1β, Ptgs-2, and iNOS. These findings demonstrate that injury stressors initiate divergent HERV responses depending on patient, HERV, and disease course and implicate HERVs as genetic elements contributing to polymorphic injury pathophysiology.

Introduction

The stress signals originating from burn injury sites are often transmitted to distant organs through various layers of both characterized and uncharacterized pathways, leading to divergent and often unpredictable clinical manifestations such as inflammatory disorder and organ failure (Fayazov et al., 2009; Kallinen et al., 2012). The mechanisms underlying the complex and polymorphic network of post-burn pathologic events have been investigated primarily by studying the relationship between burn-incited phenotypes and altered functions of genes, focusing on differential expression profiles and non-synonymous single nucleotide polymorphisms (SNPs) (Barber et al., 2004, 2006). Although substantial progress has been made in understanding the basics of local and distant response to burn injury, the vast majority of the multifactorial characteristics of the disease courses and clinical outcomes occurring in a heterogeneous population of burn patients are far from being fully grasped.

Human endogenous retroviruses (HERVs) occupy ~8% of the human genome while the entire set of protein coding genes consists of only ~3% (Lander et al., 2001; Venter et al., 2001). HERVs are reported to participate in a range of disease processes such as degeneration of oligodendrocytes, type-I diabetes mellitus, rheumatoid arthritis, and breast cancer (Conrad et al., 1997; Contreras-Galindo et al., 2008; Frank et al., 2005; Freimanis et al., 2010). In addition, the envelope (*env*) polypeptides of certain murine endogenous retroviruses (ERVs) are capable of inducing pro-inflammatory cytokines (e.g., IL-6) in macrophages (Lee et al., 2011). Burn-elicited stress signals have been found to differentially alter the expression of murine ERVs, some of which retain intact coding potentials for virion assembly, in a tissue/cell type- and time after injury-specific manner (Cho et al., 2008; Kwon et al., 2009; Lee et al., 2008; unpublished data). The ERVs, which are activated in response to burn-incited stress signals, may exert their biologic activity via their gene products and/or replication/infection (Boller et al., 2008; Holder et al., 2012; Weis et al., 2007). Alternatively, ERVs, which are integrated into genes, may affect their neighboring genes through their transcription regulatory activity and post-transcriptional modifications, including alternative splicing that leads to the generation of fusion transcripts (Feuchter-Murthy et al., 1993; Medstrand et al., 2001; Ting et al., 1992).

The human population, regardless of genetic background, is presumed to share a substantial number of HERV loci in their genomes; however, at the same time, it is anticipated that each individual has a unique genomic HERV profile. We postulate that the polymorphic HERV profiles in the genomes of a heterogeneous population of burn

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patients are closely linked to the divergent and often unpredictable disease courses and outcomes. In this study, post-burn changes in the HERV expression profiles were examined in a heterogeneous patient population, and the pathologic properties of the gene products of the burn-associated HERVs were examined.

Materials and methods

Patient population and blood collection

This study has been reviewed and approved by the Institutional Review Boards Administration of the University of California, Davis and University of Michigan, Ann Arbor in accordance with the common rule and any other governing regulations. Participants or the next of kin, caretakers, or guardians on behalf of the minors/children provided their written informed consent to participate in this study. Subjects enrolled in this study had a minimum of a 30% total body surface area burn. Detailed information regarding the patients and schedules for the blood sample collection is summarized in Table 1. Approximately 4–8 ml of blood samples was collected at several time points up to 270 days post-admission.

Semi-quantitative RT-PCR analyses of HERV expression

Buffy coat was isolated from each blood sample by centrifugation at 2000 × *g* for 10 min at room temperature. Total RNA was isolated from the buffy coat using the RNeasy Mini kit (Qiagen, Valencia, CA) with modifications, including treatment with TRIzol (Invitrogen, Carlsbad, CA) and DNase I (to remove any genomic DNA contamination). cDNA was synthesized using 100 ng of total RNA from each sample, Sensiscript reverse transcriptase (Qiagen), RNase inhibitor (Promega, Madison, WI) and an oligo-dT primer (5′-GGC CAC GCG TCG ACT AGT ACT TTT TTT TTT TTT TTT T-3′). The absence of genomic DNA contamination in the cDNA preparations was verified using the control samples without reverse transcriptase treatment. The primer sets, which were used to amplify the 3′ long terminal repeat (LTR) regions of eight different HERV families, are listed in Table 2. β-Actin was amplified as a normalization control using the primer set: 5′-CCA ACT GGG ACG ACA TGG AG-3′ and 5′-GTA GAT GGG CAC AGT GTG GG-3′. Densitometric quantitation was performed for the individual HERV amplicons using the Kodak MI system (Carestream Health, Rochester, NY). The intensity of each HERV amplicon was normalized with the matching β-actin.

Cloning and sequencing

A total of 344 HERV amplicons (from patient-1, patient-2, patient-4, and patient-11) were purified using the QIAquick Gel Extraction kit (Qiagen) and then cloned into the pGEM-T Easy vector (Promega). Three clones were picked for each amplicon, and plasmid DNAs were prepared using the QIAprep Miniprep kit (Qiagen) for sequencing analysis. Sequencing was performed at Functional Biosciences (Madison, WI). DNA sequences were analyzed using the EditSeq and MegAlign programs (DNASTAR, Madison, WI).

Multiple alignment and phylogenetic analyses of expressed HERV sequences within each HERV family

A total of 1,026 3′ LTR region sequences were obtained from the 344 HERV amplicons. To evaluate whether the expressed HERV sequences are shared among the four patients (patient-1, patient-2, patient-4, and patient-11), the LTR region sequences were subjected to alignment analyses within each HERV family using the ClustalW protocol, and phylogenetic trees were generated using the MEGA4 program (Tamura et al., 2007).

In silico mapping of HERV loci

Among the 137 and 202 unique 3′ LTR region sequences which were identified from patient-1 and patient-2, respectively, only 37 sequences were shared by both patients. The reference human genome database (Build 37.1) from the National Center for Biotechnology Information (NCBI) was surveyed for putative HERVs which share greater than 98% identity using each unique 3′ LTR region sequence as a mining probe and the Advanced Blast program. The percent identity was reduced to 95% or 90% step-wise if no hits were retrieved with the 98% identity threshold. The regions, which span 12 kb upstream and downstream from the individual LTR hits, were surveyed to identify putative HERV loci. For each putative HERV locus, the coding potentials for three genes (*gag*, *pol*, and *env*) were examined using the SeqBuilder program (DNASTAR). The open reading frames, which encode greater than 100 amino acids, were recorded and the others were denoted as defective.

Cloning of gag polypeptide coding sequences from a patient's genomic DNA

The *gag* polypeptide coding regions of two different HERVs were amplified from patient-1's genomic DNA by a two-step PCR protocol using a combination of two primer sets for each HERV to obtain locus-specificity (primer sequences are listed in Table 2). First, the 5′ LTR-*gag* regions were amplified (30 cycles) using a set of primers that span the 5′-proviral junction to the end of the *gag* coding sequence. During the second round of PCR (20 cycles), the specific *gag* coding regions (start to end) were amplified from the 5′ LTR-*gag* amplicon from the first PCR, followed by cloning into the pGEM-T Easy vector (Promega) and subcloning into the pcDNA4/HisMax expression vector (Invitrogen). All constructs were sequenced to confirm the inserts.

Table 1
Patient demographics and sample collection time points. F (female); M (male); C (Caucasian); H (Hispanic). *Blood samples from the two daughters (D1 and D2) of patient-4 were obtained as a no-burn control.

Patients	Age	Sex	Race	Sample collection: post-burn time points (hour)										
				1	2	3	4	5	6	7	8	9	10	11
Patient-1	6	F	C	48	63	135	303	643	6473					
Patient-2	13	M	C	13	25	48	61	157	325	669	2156			
Patient-3	20	M	C	74	141	316	645							
Patient-4	57	M	C	19	24	49	73	210	354	657	1261	1504	1625	3953
Patient-4:D1*	N/A	F	N/A											
Patient-4:D2*	N/A	F	N/A											
Patient-5	44	M	C	24	30	45	69	165	373	679	1665			
Patient-6	3	M	H	24	34	49	72	196	364	633	2360			
Patient-7	2	M	H	25	28	45	70	193	362	696	1416			
Patient-8	17	F	H	1	9	54	79	178	342	703				
Patient-9	12	M	H	16	26	46	68	165	333					
Patient-10	5	F	H	4	12	61	84	200	368					
Patient-11	53	F	C	96	192	360	432	936	1200	1272				