electron micrographs of sorted cells (Supplementary Table S2 online), we calculate that the total concentration of these three keratins in the average non-stem basal keratinocyte is $\sim 40\,\mu g\,\mu l^{-1}$ or 520 μM (Supplementary Table S3 online). By comparison, the total actin concentration ranges between 25 and 200 μM in various cell types (Pollard *et al.*, 2000), and is reportedly up to 900 μM in skeletal muscle cells (Jaeger *et al.*, 2009). The latter figures convey that the concentration of keratin in basal keratinocytes approximates that of actin in muscle tissue. Further, our assumptions and measurements together yield a total protein concentration of $\sim 180\,\mu g\,\mu l^{-1}$ in sorted keratinocytes (Supplementary Table S3 online), a figure that is consistent with previous reported values for mammalian cells ($50–400\,\mu g\,\mu l^{-1}$; Schnell and Turner, 2004). We note that although the soluble pool represents only 2% of the total keratin proteins in basal keratinocytes (Bernot *et al.*, 2005), the corresponding number of protein monomers (~1.9 million, Figure 2e) and concentration (~10 μM, Supplementary Table S3 online) is large, relative to the pool of most other cellular proteins. This sizable soluble pool is presumably available to sustain the remodeling of keratin filaments under steady-state conditions, and/or to fulfill nonstructural roles in the cell.

These quantitative figures are essential to a deeper understanding of keratin organization and function, and their regulation, in epidermis and related surface epithelia.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

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No Evidence for Viral Sequences in Mycosis Fungoides and Sézary Syndrome Skin Lesions: A High-Throughput Sequencing Approach

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TO THE EDITOR

The involvement of infectious, mainly (retro)viral, agents in cutaneous T-cell lymphomas (CTCL) remains a debated issue (Manzari *et al.*, 1987; Hall *et al.*, 1991; Pancake *et al.*, 1995; Bazarbachi *et al.*, 1997; Wood *et al.*, 1997; Morozov *et al.*, 2005). A recent study using a method detecting virtually all known primate T-cell lymphotropic viruses (PTLVs) failed to identify any PTLV-related sequence in a series of CTCL patients, confirming previous negative reports (Courgnaud *et al.*, 2009). However, the issue of an implication of a new or already known virus is

Abbreviations: CTCL, cutaneous T-cell lymphomas; HTS, high-throughput sequencing; MCPyV, Merkel cell polyomavirus; MF, mycosis fungoides; PTLV, primate T-cell lymphotropic virus

still pending and deserves additional investigations. In this perspective, the recently developed and highly powerful high-throughput sequencing (HTS) approach is of particular interest, as it may identify new, currently unknown nucleic sequences in biological samples that may be related to infectious agents and viruses in particular. A major recent achievement of this ''broad approach'' method, free of any underlying hypothesis regarding a particular subset of infectious agents, is the identification of Merkel cell polyomavirus (MCPyV) in an aggressive neuroendocrine skin tumor (Feng *et al.*, 2008; Shuda *et al.*, 2008). However, no such experiment has been conducted in CTCL to date.

To address this issue, the whole transcriptome of cutaneous lesions from six patients with CTCL (three with stage Ia–IIa mycosis fungoides (MF) and three with Sézary syndrome with blood involvement fulfilling ISCL criteria) was analyzed for the presence of viral transcripts from known or unknown species by HTS in a pilot study. More detailed information about the investigated patients is available as Supplementary Material online. To ensure results homogeneity, blood sample from SS patients was not analyzed. Written informed consent was obtained from all patients and the experiments were conducted in accordance with the Declaration of Helsinki Principles. Total RNA was extracted with Trizol, retrotranscribed, and randomly amplified as recently described (Cheval *et al.*, 2011). High-throughput sequencing was subcontracted to GATC Biotech AG (Konstanz, Germany). The single end sequencing was performed with an Illumina (San Diego, CA) GA II on two channels and was conducted with an average depth of 5.8 million reads per sample and a length of 96 nucleotides per read. Sequences were first selected or trimmed according to their quality scores. The human genome was filtered with SOAPaligner (http://soap.genomics.org.cn) using the *Homo sapiens* hg19 reference. This host filtering step eliminated an average of 91.6% reads per sample. Remaining reads (ranging from 249,570 to 776,095) were assembled in contigs using CLC Genomics Workbench (http://www.clcbio.com). On average, 5,668 contigs with a length of 100 nucleotides or more were generated (with a minimum of 830 contigs and a maximum of 15,770 per sample). A comparison of the single reads and contigs with available genomic and taxonomic data was made on the generalist nucleotidic (nt) and proteic (nr) databases maintained locally. The aforementioned databases were scanned using the BlastN and BlastX algorithms provided by Paracel Blast (Striking Development, Los Angeles, CA), a software capable of executing searches on multiple nonshared memory processors simultaneously. Binning (or taxonomic assignment) was based on the best hit among reads with a significant *e*-value (below 10^{-3}). Nonassignated sequences corrresponded to 2–13% of the contigs (maximal size 922 nt) and 5–22% of the reads.

As disclosed in Table 1, no known viral transcript was detected in CTCL cutaneous lesions, except for rare hits against endogenous retroviruses that are common in all tissue samples we have examined till now, regardless of their origin (not shown). Moreover, no

Table 1. Viral and bacterial contigs derived from the transcriptome of six patients

	Number of contigs	Average contig identity (%)	Number of assembled reads
Eukaryotic viruses			
Human endogenous retrovirus	2	99.6	5
Human endogenous retrovirus K	1	98.0	2
Human endogenous retrovirus HERV-K (I)	1	97.3	5
Bacteria			
Actinobacteria	19	47.9	317
Bacteroidetes	44	51.1	1,236
Candidate division WWE1; *Candidatus Cloacamonas*	1	26.3	13
Chlamydiae	2	67.3	16
Chlorobi	1	48.4	6
Chloroflexi	1	40.9	14
Cyanobacteria	40	67.5	12,837
Deferribacteres	4	53.1	23
Deinococcus thermus	2	35.7	208
Elusimicrobia	1	66.3	3
Environmental samples	10	90.9	1,099
Firmicutes	155	59.2	2,047
Fusobacteria	11	52.0	91
Lentisphaerae	1	39.4	18
Planctomycetes	4	44.1	18
Proteo.Alphaproteobacteria	22	53.3	199
Proteo.Betaproteobacteria	19	56.3	1,471
Proteo.Deltaproteobacteria	4	59.5	16
Proteo.Epsilonproteobacteria	24	55.0	322
Proteo.Gammaproteobacteria	85	59.8	1,770
Spirochetes	21	65.0	188
Tenericutes	38	69.8	15,312
Thermotogae	3	81.0	32
Verrucomicrobia	1	38.2	132

singleton (nonassembled read) exhibited a significant homology with a known virus (not shown), whereas singletons from housekeeping genes (β-actin, β2 microglobulin) were detected in each of the six samples (not shown). A number of bacterial transcripts of various origins were also detected, partially related to a contamination from the skin or the environment as recently described by us in a metagenomic study of skin surface (Foulongne *et al.*, 2012).

HTS is a highly comprehensive method based on random sequencing of nucleic acids present in a given sample and likely to ensure the detection of every possible sequence from microorganisms present in the target tissue, either previously known or not. This pilot study is, to our knowledge, a previously unreported attempt to use HTS in search for infectious agents and, more particularly, for viral sequences in CTCL. We have chosen to sequence all RNAs, without any selection for polyA+ RNAs, as a hallmark of the presence of all pathogens including bacteria. As recently described (Cheval *et al.*, 2011), our pipeline is able to detect viruses present in databases with a level of sensitivity roughly equivalent to quantitative PCRs and to acquire full-length genomes (Bouquet *et al.*, 2012; Foulongne *et al.*, 2012). It can also identify unknown viruses (Sauvage *et al.*, 2011a, 2011b) even when the viral species defines a new genus within a family. This powerful procedure is thus particularly suited to situations where involvement of an infectious agent is suspected but none has been detected/found. In the present study, HTS yielded no sequence corresponding to a known or unknown viral agent. More particularly, no sequence from the newly described polyomaviruses was identified, but most of them are probably of limited distribution except for MCPyV and a random effect cannot be ruled out. Nevertheless, methodological limitations may have reduced the import of these negative results. First, this study was conducted in a limited number of patients all originating from a particular geographical area (southern France) and no definitive conclusion can be drawn. Different results might be obtained in other areas by analogy, with observations made for MCPyV in MCC (Garneski *et al.*, 2009). Second, the selected patients were not representative of all subsets of CTCL as folliculotropic MF and CD30+ lymphoproliferative disorders were not represented. Despite these limitations, these data clearly contribute to a body of evidence that argues against infectious, more particularly viral agents being an etiologically relevant general characteristic in these patients, owing to the high investigational power of the molecular tool. It remains to determine whether or not this negative result is shared by all subsets of CTCL, especially in folliculotropic MF, as follicles are well-known sanctuaries for diverse viruses such as HPV.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

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