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No Viral Transcripts Associated with Folliculotropic Mycosis Fungoides Using a High Throughput Sequencing Approach

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Written informed consent was obtained from all patients and the experiments were conducted in accordance with the ICH GCP. Fresh-frozen 4-mm skin biopsies from characteristic skin lesions located on the trunk or lower limbs were obtained in 6 male patients (mean age 51.5 years, range 26–76 years) diagnosed with FMF stage IA (2 patients), IB (3 patients) and IIB (1 patient).

Total RNAs were extracted with Trizol from skin biopsies, reverse transcribed and randomly amplified to high molecular weight DNA, as described previously (7). Library preparations and sequencing with an Illumina HiSeq2000 sequencer were outsourced to DNA Vision (Charleroi, Belgium). HTS analysis for the presence of viral RNA (e.g. transcripts of DNA viruses and/or genome/transcripts of RNA viruses) was conducted as reported previously (8). Endogenous retroviral sequences different from those harboured by the prototypal hG19 human sequence are not reported, because they are part of the human DNA: thus, they are different from exogenous viruses targeted by this method.

RESULTS AND DISCUSSION

No known or unknown viral sequences could be detected by the powerful methods used in this study. These negative results first raise the issue of the sensitivity of RNA-based HTS, reminiscent of the very low viral load of MCPyV, which was established at less than 0.5 copies/cell, and 0.002–12.467 copies/beta-globin gene copy in previous studies (5, 6). However, it must be pointed out that the same method has already succeeded in detecting viral sequences shown by RT-qPCR to be in very low amount, which is an indirect evidence of its high level of sensitivity (7). Furthermore, the relevant negative predictive value of our results regarding the presence of viral transcripts is attested by the coverage of rare cellular transcripts (Table S1).

Although based on only 6 skin samples, these preliminary data do not support the hypothesis that RNA viruses are significantly involved in FMF. Similarly, the lack of detection of transcripts from DNA viruses strongly suggests that they are genuinely absent or that the level of transcription is below the sensitivity threshold of the method. However, DNA viruses might be involved in FMF through other pathways than viral antigen expression, such as insertion-driven mutations of the host cell genome by few bases or full viral genome, as non-transcribed sequences that would not have been detected by the powerful methods used in this study. These negative results first raise the issue of the sensitivity of RNA-based HTS, reminiscent of the very low viral load of MCPyV, which was established at less than 0.5 copies/cell, and 0.002–12.467 copies/beta-globin gene copy in previous studies (5, 6). However, it must be pointed out that the same method has already succeeded in detecting viral sequences shown by RT-qPCR to be in very low amount, which is an indirect evidence of its high level of sensitivity (7). Furthermore, the relevant negative predictive value of our results regarding the presence of viral transcripts is attested by the coverage of rare cellular transcripts (Table S1).

Altogether, these preliminary results and considering that fMF displays specific clinical characteristics, a deep lymphocytic infiltrate aggressing hair follicles, and a possibly less favourable outcome than MF, interestingly, the presence of Merkel cell polyomavirus (MCPyV) DNA was identified by real-time PCR in 50–75% vs. 13% of patients with FMF and CMF, respectively, in 2 series, using both formalin-fixed paraffin-embedded skin biopsies (5) and fresh-frozen tissues (6), with a higher viral load in FMF compared with CMF, T-cell mediated benign skin infiltrates (psoriasis, eczemas, etc.), or healthy individuals’ skin biopsies (6). Owing to these preliminary results and considering that follicles might represent potential reservoirs for various infectious agents, RNA-based HTS was performed on skin biopsies from 6 patients with FMF.

METHODS

Written informed consent was obtained from all patients and the experiments were conducted in accordance with the ICH GCP. Fresh-frozen 4-mm skin biopsies from characteristic skin lesions located on the trunk or lower limbs were obtained in 6 male patients (mean age 51.5 years, range 26–76 years) diagnosed with FMF stage IA (2 patients), IB (3 patients) and IIB (1 patient).

Total RNAs were extracted with Trizol from skin biopsies, reverse transcribed and randomly amplified to high molecular weight DNA, as described previously (7). Library preparations and sequencing with an Illumina HiSeq2000 sequencer were outsourced to DNA Vision (Charleroi, Belgium). HTS analysis for the presence of viral RNA (e.g. transcripts of DNA viruses and/or genome/transcripts of RNA viruses) was conducted as reported previously (8). Endogenous retroviral sequences different from those harboured by the prototypal hG19 human sequence are detected by this method. However, they are not reported, because they are part of the human DNA: thus, they are different from exogenous viruses targeted by this method.

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REFERENCES