

BRIEF REPORT

Malignant Transformation of *Hymenolepis nana* in a Human Host

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SUMMARY

Neoplasms occur naturally in invertebrates but are not known to develop in tapeworms. We observed nests of monomorphic, undifferentiated cells in samples from lymph-node and lung biopsies in a man infected with the human immunodeficiency virus (HIV). The morphologic features and invasive behavior of the cells were characteristic of cancer, but their small size suggested a nonhuman origin. A polymerase-chain-reaction (PCR) assay targeting eukaryotes identified *Hymenolepis nana* DNA. Although the cells were unrecognizable as tapeworm tissue, immunohistochemical staining and probe hybridization labeled the cells in situ. Comparative deep sequencing identified *H. nana* structural genomic variants that are compatible with mutations described in cancer. Invasion of human tissue by abnormal, proliferating, genetically altered tapeworm cells is a novel disease mechanism that links infection and cancer.

H. NANA, THE DWARF TAPEWORM, IS THE MOST COMMON HUMAN TAPEWORM; up to 75 million persons are estimated to be carriers, and the prevalence among children is as high as 25% in some areas.¹ Infections are typically asymptomatic. *H. nana* is unique among tapeworms in that it can complete its life cycle in the small intestine, without the need for an intermediate host. Such autoinfection can persist for years and lead to a high parasite burden, particularly in immunocompromised hosts. Infections are generally limited to the gastrointestinal tract, where eggs released in the small bowel by adult tapeworms hatch. The embryos (oncospheres) invade the host intestinal villi, where they are transformed into larvae (cysticercoids) before breaking out and reattaching to the mucosal lining.

Extraintestinal *H. nana* infections are rare. Here we describe a man with HIV infection in whom samples from lymph-node and lung biopsies revealed monomorphic, undifferentiated cells. The proliferative cells had overt features of a malignant process, but their small size suggested a nonhuman origin. Proliferation in the immunosuppressed host may have allowed somatic mutations to accumulate in the *H. nana* stem-cell population, ultimately leading to malignant transformation.

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CASE REPORT



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In January 2013, a 41-year-old man in Medellín, Colombia, presented with fatigue, fever, cough, and weight loss of several months' duration. He had received a diagnosis of HIV infection in 2006 and was nonadherent to therapy; the most recent CD4 cell count was 28 per cubic millimeter, and the viral load was 70,000 copies per milliliter. Stool examination revealed *H. nana* eggs and *Blastocystis hominis* cysts. Computed tomographic imaging showed lung nodules ranging in size from 0.4 to 4.4 cm (Fig. 1A and 1B), as well as liver and adrenal nodules and cervical, mediastinal, and abdominal lymphadenopathy. Excisional biopsy of a cervical lymph node and core-needle biopsy of the lung were performed. The Centers for Disease Control and Prevention (CDC) was initially consulted by means of tele-diagnosis, with digital images sent to the Web-based DPDx diagnostic laboratory; paraffin-embedded tissues were subsequently submitted to the CDC. The patient received three doses of albendazole as empirical treatment, and anti-retroviral medications were reinstated. The disease progressed, and a second cervical lymph-node biopsy was performed in April 2013, with fresh tissue sent to the CDC for evaluation.

The lymph nodes were grossly abnormal, solid, nodular masses (Fig. 1C), from which a touch preparation showed small, atypical cells with scant cytoplasm and prominent nucleoli (Fig. 1D). Histologic examination showed effacement of normal architecture by irregular, crowded nests of small, atypical cells (Fig. 1E). Syncytia containing atypical nuclei were present at the periphery of the nests (Fig. 1F). The individual cells had scant cytoplasm and measured 5 to 6 μm in diameter (slightly smaller than a human red cell), with nuclei that were approximately 2 to 3 μm in diameter. Occasional cells were markedly enlarged, with pleomorphic nuclei containing multiple nucleoli (Fig. 1G). Mitotic figures, angiolymphatic invasion, and necrosis were also observed. Similar cells were present in the sample from the core-needle biopsy of the lung (see Fig. S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org). Transmission electron microscopy showed that the cytoplasm was ribosome-rich and contained scattered mitochondria (Fig. 1H). Other than syncytia formation, no feature of dif-

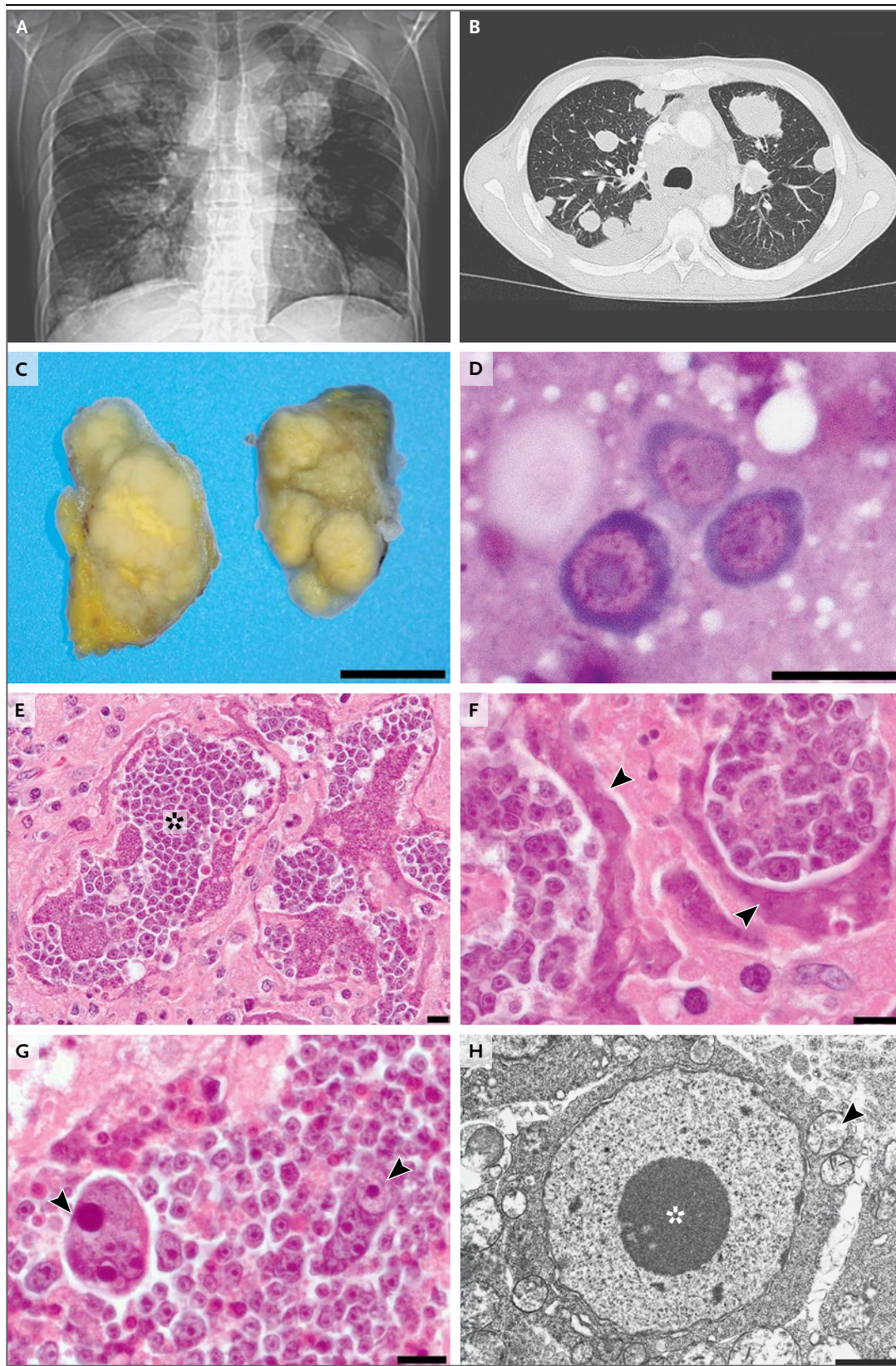
Figure 1 (facing page). Radiographic and Pathological Features of Malignant *Hymenolepis nana*.

Anteroposterior and axial CT scans in Panels A and B, respectively, show the presence of lung nodules. Panel C shows a biopsy specimen from a cervical lymph node containing firm, solid masses. Panel D shows small, atypical cells in an air-dried lymph-node touch preparation stained with Diff-Quik. Hematoxylin and eosin staining of a lymph-node histologic section shows invasive, irregular nests of proliferative cells (asterisk) in Panel E, monomorphic cellular features and multinucleated syncytia (arrowheads) on a higher-power field in Panel F, and cytologic atypia with occasional large, pleomorphic nuclei and multiple nucleoli (arrowheads) in Panel G. The transmission electron micrograph in Panel H shows ribosome-rich cytoplasm with scattered mitochondria (arrowhead) and a nucleus with a conspicuous central nucleolus (asterisk). Scale bars correspond to 1 cm in Panel C, 10 μm in Panels D through G, and 1 μm in Panel H.

ferentiation, including formation of microvilli, was seen (Fig. S2 in the Supplementary Appendix). Immunohistochemical staining of these cells was negative for human cytokeratin and vimentin (often expressed in cancer cells), as well as for free-living amebas.

This case posed a diagnostic conundrum. The proliferative cells had overt features of a malignant process — they invaded adjacent tissue, had a crowded and disordered growth pattern, and were monomorphic, with morphologic features that are characteristic of stem cells (a high nucleus-to-cytoplasm ratio) — but the small cell size (<10 μm in diameter) suggested infection with an unfamiliar, possibly unicellular, eukaryotic organism. Infection with a plasmodial slime mold (phylum, Amoebozoa; class, Myxogastria) was considered because of the prominent syncytia formation. Although many cestode tissues are syncytial — notably, their tegument — a tapeworm infection was initially considered less likely because of the primitive appearance of the atypical cells, the complete absence of architecture that was identifiable as tapeworm tissue, and the rarity of previously reported cases of invasive cestodiasis.^{2,3}

During our laboratory investigations, the lesions of the lung, liver, and adrenal glands remained stable, but the lymph nodes (particularly in the neck) increased to a maximum of 5 cm in diameter, and over the course of 4 months, the patient's clinical condition deteriorated. The patient was receiving tenofovir for the treatment of



HIV infection and amphotericin B for histoplasmosis, and renal failure developed. The patient declined hemodialysis, and the delivery of palliative care was begun in May 2013. A molecular diagnosis was provided 72 hours before he died, and specific treatment was not attempted. A post-mortem examination was not performed. Before he died, the patient provided written informed consent for studies to be performed and for publication of the results.

METHODS

CELL CULTURE

We performed cell culture with the use of culture methods for free-living amebas and Myxogastria, including agar plates and tissue cultures of human lung fibroblasts. (The complete details of these and other methods are provided in the Supplementary Appendix.)

PCR ASSAYS

DNA was extracted from the tissue specimens with the use of standard methods. For the initial molecular studies, we used Myxogastria and pan-fungal PCR and sequencing assays targeting the 18S ribosomal RNA (rRNA) gene and internal transcribed spacer regions (525 bp and approximately 650 bp, respectively).^{4,5} A subsequent cestode-specific PCR assay targeted a 206-bp fragment of the 18S rRNA gene,² and a hymenolepidid-species-specific PCR assay targeted a 391-bp fragment of the gene encoding cytochrome *c* oxidase (COI)⁶ (see the Methods section and Table S1 in the Supplementary Appendix).

IMMUNOHISTOCHEMICAL STUDIES AND IN SITU HYBRIDIZATION

Immunohistochemical studies were performed with the use of a polymer-based indirect immunoperoxidase detection system and fast-red chromogen. Polyclonal rabbit antiserum against *Taenia solium* GP50 antigens was used at a 1:250 dilution, which labels the *T. solium* cysticercus bladder epithelium, *H. nana* adult tegument, and *H. diminuta* cysticercoids (see the Methods section and Fig. S3 in the Supplementary Appendix). Digoxigenin-labeled DNA probes targeting a 206-bp fragment of the *H. nana* 18S rRNA gene and a 224-bp conserved area of human Alu sequences were prepared with the use of standard methods (see the Methods section and Table S1 in the Supplementary Appendix).

PHYLOGENETIC ANALYSIS

The product of the PCR assay of the *H. nana* COI gene described above was bidirectionally sequenced, and a repeat PCR assay and sequencing reaction were used to confirm the results (National Center for Biotechnology Information [NCBI] number, KT362138). Together with *H. nana* sequences available in GenBank, nucleotide data were aligned with the use of MUSCLE software, and a phylogenetic tree was estimated by means of the neighbor-joining method (MEGA software, version 5.2).

GENOMIC SEQUENCING AND COMPARATIVE ANALYSIS

DNA from the specimen from the patient's second cervical lymph-node biopsy and DNA from cryopreserved *H. nana* reference-strain specimens were used to construct sequencing libraries and were sequenced with the use of the MiSeq system (Illumina). The contaminating human reads were filtered with the use of CLC Genomics Workbench, version 7.0.4 (CLC bio), by mapping to the reference human genome (Genome Reference Consortium human genome build 37). Next, the remaining sequencing reads (NCBI number, SRP061937) were mapped to the unannotated *H. nana* laboratory reference-strain genome (<http://parasite.wormbase.org>).

The relatively incomplete genome coverage allowed for qualitative analysis of copy-number and structural variants.⁷ Insertional mutations were detected on the basis of nearby left and right sequencing reads with abrupt changes in sequence (split reads). Deletions, inversions, and point mutations were not formally examined. Genes were predicted by means of homology with the use of the annotated *H. microstoma* genome.^{8,9} The complete details are provided in the Methods section in the Supplementary Appendix.

RESULTS

CELL CULTURE

Cell culture was attempted from the fresh tissue. However, no growth occurred after 4 weeks of incubation.

MOLECULAR IDENTIFICATION AND CONFIRMATION

We performed Myxogastria and panfungal PCR assays in an attempt to target an unknown eukaryote, but these assays unexpectedly identified *H. nana* with 99% sequence identity. The presence of *H. nana* DNA in the specimen was confirmed

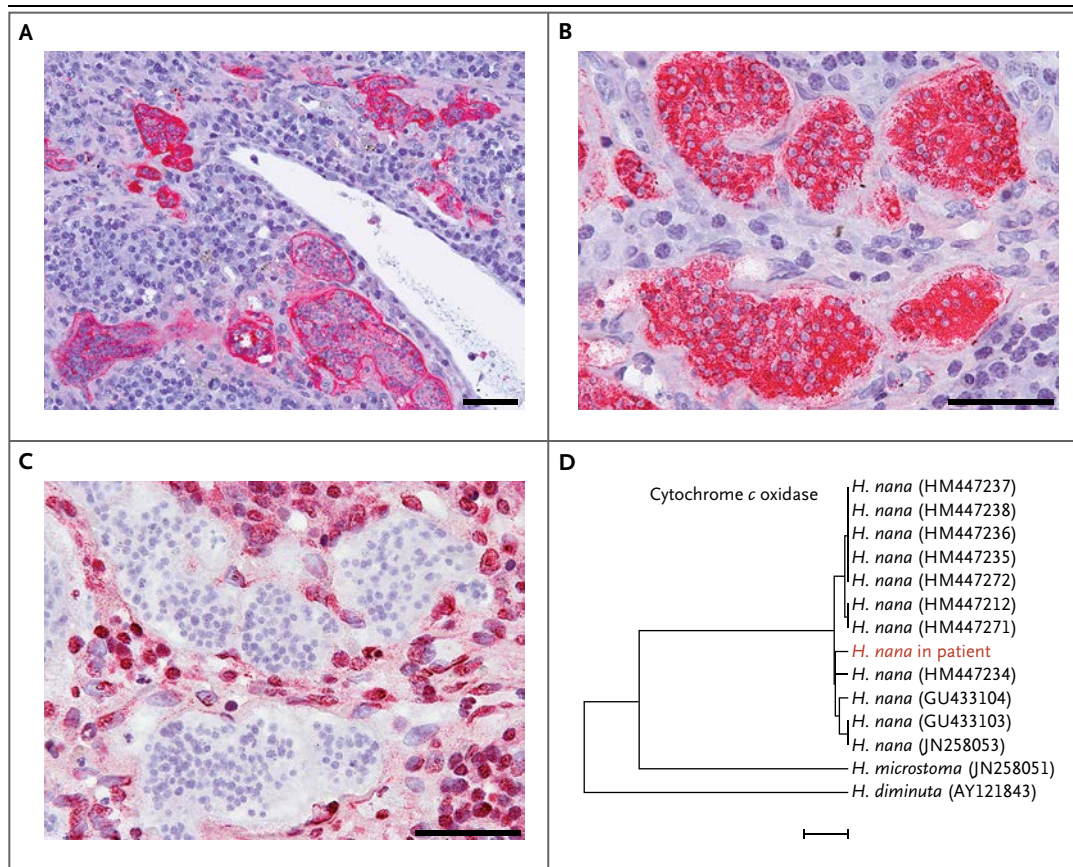


Figure 2. Confirmation of *H. nana* Infection.

Proliferative cells are labeled by means of immunohistochemical staining with the use of a cross-reactive polyclonal antiserum against *Taenia solium* GP50 antigens, shown in Panel A, and in situ hybridization with the use of a cestode 18S ribosomal DNA probe, shown in Panel B, with an absence of proliferative-cell labeling on in situ hybridization with a human Alu probe, which labels the surrounding human cells, shown in Panel C. Scale bars in Panels A, B, and C correspond to 50 μ m. Panel D shows a phylogenetic analysis of the 391-bp *H. nana* CO1 nucleotide sequence in the patient (KT362138), together with all available *H. nana* sequences; the scale bar corresponds to a genetic distance of 0.02 substitutions per site.

by cestode- and hymenolepidid-species-specific PCR testing and sequencing. The molecular findings were surprising, since there was no recognizable tapeworm tissue architecture; thus, to confirm that the cells originated from a tapeworm, we performed immunohistochemical studies and in situ hybridization, which localized cestode antigen and nucleic acid markers (Fig. 2A, 2B, and 2C).

PHYLOGENETIC AND MITOCHONDRIAL DNA ANALYSIS

The CO1 sequence obtained from the patient was grouped within the clade of known *H. nana* sequences (Fig. 2D). An unexpected feature of the patient-derived sequence was the presence of three single-nucleotide insertions within a span of 12 bp in a highly conserved domain (NCBI

Conserved Domain Database, cd01663) (Fig. S4 in the Supplementary Appendix), which was compatible with a deleterious mutation.

COMPARATIVE GENOMIC ANALYSIS

Deep sequencing of the specimen from the patient generated 10.2 million 150-bp, paired-end reads. Removal of contaminating human sequences resulted in 1.7 million remaining reads, of which 1.4 million mapped onto the *H. nana* reference genome, with 53% coverage, at an average coverage of 2.4 times per base (excluding zero-coverage regions). From the *H. nana* control specimen, 7.1 million reads were mapped, with 93% coverage, at an average coverage of 7.0 times per base.

Amplifications were detected by evaluating genomic regions that had increased coverage in

the biopsy specimen as compared with the control specimen. Both exons of the gene encoding lysosome-associated membrane protein 2 (*LAMP2*), predicted on the basis of homology with the annotated *H. microstoma* genome,^{8,9} were present in two of the top four overrepresented contigs and scaffolds (increased by a factor of 13 in one case and 25 in the other) (Fig. 3A, 3B, and 3C). Multiple split-read sequences within these conserved regions were present in the specimen from the patient but not in the control specimen, findings that are compatible with complex genomic rearrangement and amplification. Similar alterations were seen in other regions without predicted homologous genes.

Insertion-site analysis identified six insertional mutations (Table S2 in the Supplementary Appendix), of which five were associated with protein-coding genes predicted on the basis of homology with *H. microstoma*. The predicted genes were the transcriptional regulator *PHF10/BAF45a* (implicated in development), the gene encoding protein kinase *ULK2* (implicated in autophagy), a putative C2H2 zinc-finger transcription factor, the gene encoding the actin-binding protein *IPP*, and a gene encoding a novel protein. Mammalian homologues of three of these genes — *PHF10*, *ULK2*, and *IPP* — have been studied in cancer (Table S3 in the Supplementary Appendix). Furthermore, four of the five *H. microstoma* genes are differentially expressed between larval and adult tissues (Fig. 3D).

DISCUSSION

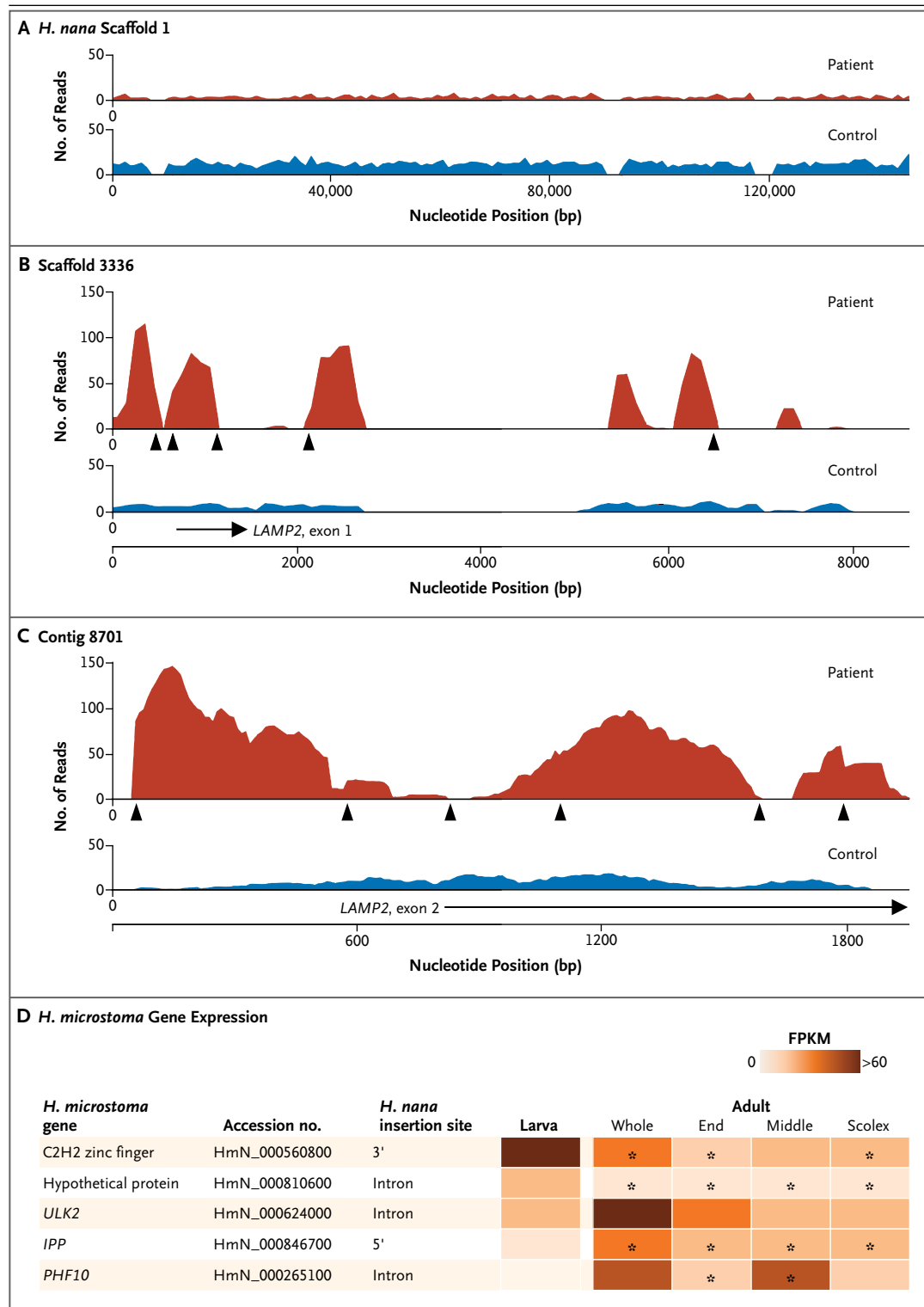
Although extraintestinal *H. nana* infections are rare, cysticercoids have been reported in whole-blood preparations from glucocorticoid-treated children,¹⁰ and a case of fatal invasive *H. nana* infection with atypical morphologic features was described in an HIV-positive man.^{2,3} *H. nana* is known to develop abnormal, enlarged, ballooned cysticercoids in immunosuppressed mice.^{11,12} Normal tapeworm development probably requires signals from immune responses of normal hosts,¹³ as is further suggested by in vitro cultures of hydatid tapeworms.¹⁴ Atypical proliferative infections with other tapeworm species have also been reported in humans^{2,15} and in other animals, including orangutans¹⁶ and cats.¹⁷ In contrast to the current case, all previously reported cases of invasive cestodiasis showed recognizable metazoan tissue architecture.

Figure 3 (facing page). Structural Genomic Alterations in *H. nana*.

Panel A shows sequencing coverage in the biopsy specimen from the patient as compared with the *H. nana* control, along the length of *H. nana* scaffold 1. Panels B and C show complex copy-number alterations in conserved genomic regions containing both exons 1 and 2 of *LAMP2*. Arrowheads indicate split-read sequences that are present in the specimen from the patient but not in the control specimen, findings that are compatible with chromosomal breakpoints. Panel D shows insertion mutation–associated genes, with *H. microstoma* gene expression data comparing expression levels in larvae with those in various adult tissues; asterisks indicate expression levels in adult tissues that differ significantly from the level of expression in larval tissue.⁸ FPKM denotes fragments per kilobase of transcript per 1 million mapped reads.

Neoplastic cellular proliferations occur in invertebrates^{18,19} and have been described in free-living flatworms,²⁰ but to our knowledge, such proliferations have not previously been documented in multicellular parasites, including tapeworms. In this case, proliferating cells with monomorphic morphologic features, cytologic atypia, and genetic alterations fulfill working definitions of neoplasia, and the presence of tissue invasion and metastasis is characteristic of a malignant process. Although the genomic variability of *H. nana* in human populations is unknown, the observed genetic alterations are compatible with mutations seen in mammalian cancer. These include deleterious mitochondrial gene mutations (which occur in up to 70% of colorectal carcinomas²¹), complex genomic rearrangements, and a predominance of intragenic as compared with intergenic insertional mutations.²² *H. microstoma* homologues of the insertion-associated genes appear to be functionally important and are differentially expressed in larval tissues. These data suggest that the insertional mutations we observed were nonrandom, were likely to have been selected for during cellular proliferation, and may promote cellular growth.

In contrast to mammalian stem cells, pluripotent stem cells in flatworms (called neoblasts) are the only cells with proliferative capacity, and differentiated cells do not divide.²³ Cestode stem cells were recently characterized in detail in the hydatid tapeworm *Echinococcus multilocularis*.²⁴ The mitotic activity, small size, ribosome-rich cytoplasm, comparatively large nuclei, and prominent nucleoli of the cells from the patient described



here are entirely consistent with tapeworm stem cells. We hypothesize that continued proliferation in the immunosuppressed host allowed somatic mutations to accumulate in the *H. nana*

stem-cell population, ultimately leading to malignant transformation.

Malignant transformation of *H. nana* may be misdiagnosed as human cancer, particularly in

underdeveloped countries in which HIV and *H. nana* infections are widespread. Typical gastrointestinal *H. nana* infection is treated with praziquantel or nitazoxanide, and albendazole is the drug of choice for tissue-invasion stages of larval cestodes. However, the efficacy of albendazole against clonal proliferations of tapeworm stem cells as opposed to whole organisms is questionable. Preliminary data from in vitro studies using cultured cestode stem cells²⁵ suggest that albendazole is ineffective (Brehm K: personal communication). Invasive *H. nana* cellular proliferations may therefore present a new challenge to therapy.

Infectious agents, such as human papilloma-virus and *Schistosoma haematobium*, contribute to human cancer worldwide. Transmissible clones of cancer cells circulate naturally within populations of Tasmanian devils and domestic dogs.²⁶

In humans, cancer cells are infrequently transmitted through organ transplantation or from mother to fetus during pregnancy. Human disease caused by parasite-derived cancer cells is a novel finding. Multicellular parasites that live in host tissue generally possess cellular mechanisms for host tissue invasion and immune evasion; these mechanisms could potentially be co-opted during malignant transformation within the host. The host–parasite interaction that we report should stimulate deeper exploration of the relationships between infection and cancer.

The views expressed in this article are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Disclosure forms provided by the authors are available at NEJM.org.

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