

# Cellular Transcriptome Analysis Reveals Differential Expression of Pro- and Antiapoptosis Genes by Varicella-Zoster Virus-Infected Neurons and Fibroblasts

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**Transcriptional changes following varicella-zoster virus (VZV) infection of cultured human neurons derived from embryonic stem cells were compared to those in VZV-infected human foreskin fibroblasts. Transcription of 340 neuronal genes significantly altered by VZV infection included 223 transcript changes unique to neurons. Strikingly, genes inhibiting apoptosis were upregulated in neurons, while proapoptotic gene transcription was increased in fibroblasts. These data are a basis for discovery of differences in virus-host interactions between these VZV targets.**

The alphaherpesvirus varicella-zoster virus (VZV) radically modifies the transcription of cellular genes upon infection (1, 2). However, while the transcriptional changes seen in lytic VZV infection of several cell types have been studied and partly delineated (1–4), changes in neurons have not been studied. Neurons host the VZV latent state after a primary infection, from which virus may reactivate after decades to cause herpes zoster. The strict human specificity of VZV precludes transcriptome analysis in animal neuron model systems. However, recently introduced *in vitro* human neuron systems, including neurons made from human embryonic stem cells (hESC), now permit comparison of cellular transcriptome changes following VZV infection to those in non-neuronal cells.

Neurons generated from hESC line H9 and human perinatal foreskin fibroblasts (HFF) were prepared and grown as detailed

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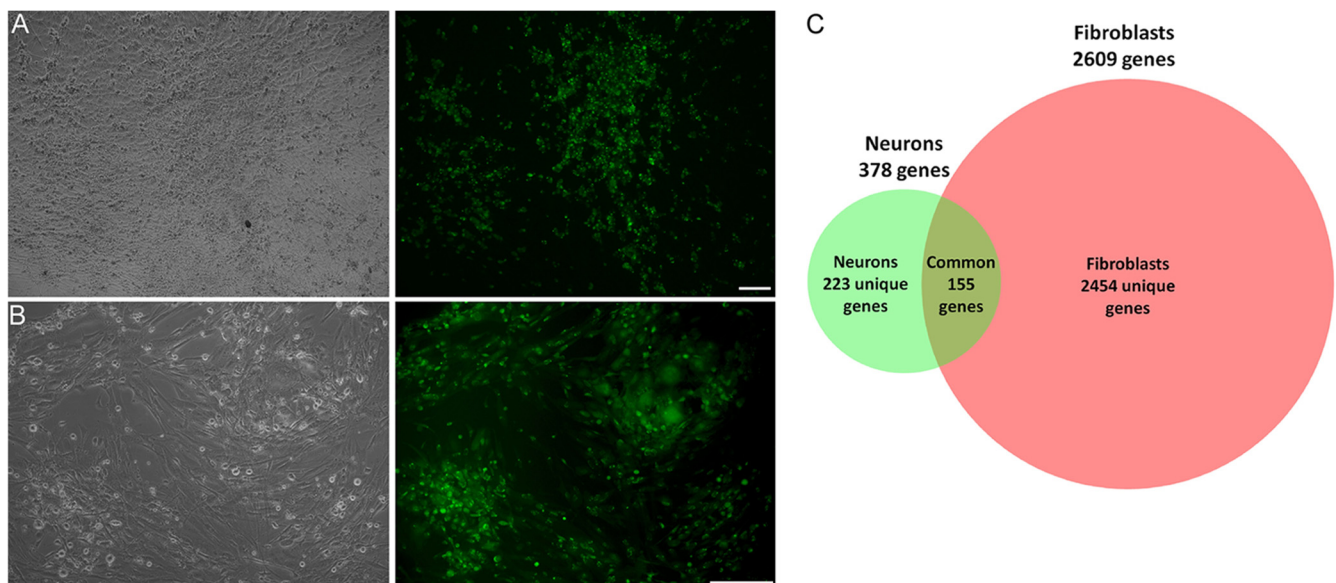
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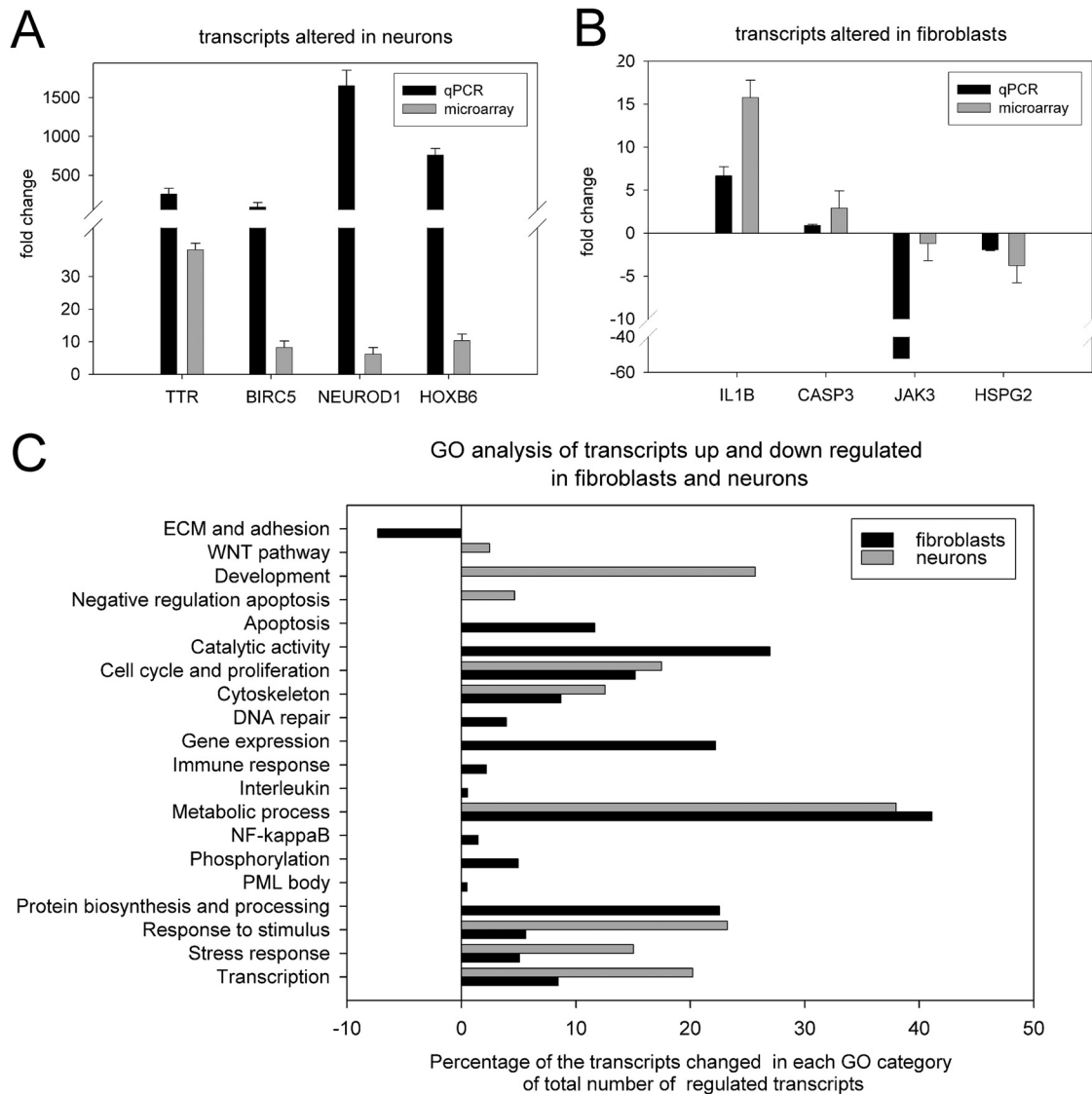
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**FIG 1** (A and B) Infection of HFF and hESC-derived neurons with GFP-expressing VZV. VZV-infected hESC-derived neurons (A) and VZV-infected human foreskin fibroblasts (B) at time of harvest for RNA. Left panels, phase-contrast micrographs; right panels, fluorescence micrographs. Bars, 100  $\mu$ m. (C) Venn diagram summarizing gene expression changes in neurons and fibroblasts following infection with VZV. The red circle represents the expression of 2,609 genes altered in infected fibroblasts compared to uninfected fibroblasts; 1,654 genes were upregulated and 955 genes were downregulated. Out of these genes, 2,474 genes were altered specifically in fibroblasts. The green circle represents the expression of 378 genes altered in infected neurons compared to uninfected neurons; 340 genes were upregulated and 38 genes were downregulated. Out of these, 223 genes were altered only in neurons.



**FIG 2** (A and B) Confirmation of transcription changes by quantitative RT-PCR (qPCR) for four genes that were altered in neurons (A) and fibroblasts (B) as a result of VZV infection. (C) Gene ontology analysis of transcription changes in neurons and fibroblasts in response to VZV infection. All GO changes were significant with a Benjamini  $P$  value of  $<0.05$ . The bars indicate the genes changed as percentages of the total number of regulated genes in each GO category. Black bars depict fibroblast transcripts, and gray bars depict neuronal transcripts.

previously (5). Neurons (minimum of 2 weeks of terminal differentiation) and fibroblasts at 95% confluence were infected with cell-free preparations of recombinant VZV (6) expressing green fluorescent protein (GFP) as a fusion to the ORF23 capsid protein (7) at 10,000 PFU per well of a 24-well plate. RNA was prepared from 80% to 100% GFP-positive infected cultures (Fig. 1A and B) before plaque formation in fibroblasts and development of obvious cytopathology in neurons. RNA was hybridized to Agilent Human GE 4x44K v2 microarray transcriptome microarrays (G2519F-026652 covering 34,127 transcripts) and analyzed using standard procedures. Two biological replicates and two technical replicates were performed. Select changes were confirmed with quantitative reverse transcription-PCR (qRT-PCR), using Sybr green in-house, as well as blindly by a commercial service (Syd Labs, CA, USA).

Using a cutoff of 2-fold changes with a significance of 0.05, the expression of 378 genes was modified in hESC-derived neurons,

with 340 genes (90%) upregulated and only 38 transcripts (10%) downregulated by VZV infection (Fig. 1C). Similar skewing of transcription changes toward upregulation has been reported in neurons infected with other alphaherpesviruses (reviewed in reference 8). More significant changes in transcription followed VZV infection of HFF, with 2,609 significant changes (Fig. 1C), of which 1,654 (63%) showed upregulation and 955 (36%) showed downregulation. Many transcriptional changes were the same as those reported in array studies for fibroblasts detailed previously (1, 2). Of note is that 223 transcriptional changes were specific to neurons (Fig. 1C; see also Tables S1 to S3 in the supplemental material). Quantitative RT-PCR analyses of 8 select genes (4 with RNA from fibroblasts and 4 with RNA from neurons) all confirmed changes in expression identified by the microarray studies (Fig. 2A and B).

Gene ontology (GO) analysis using DAVID (Fig. 2C) revealed that transcription of genes in 17 functional categories was signif-

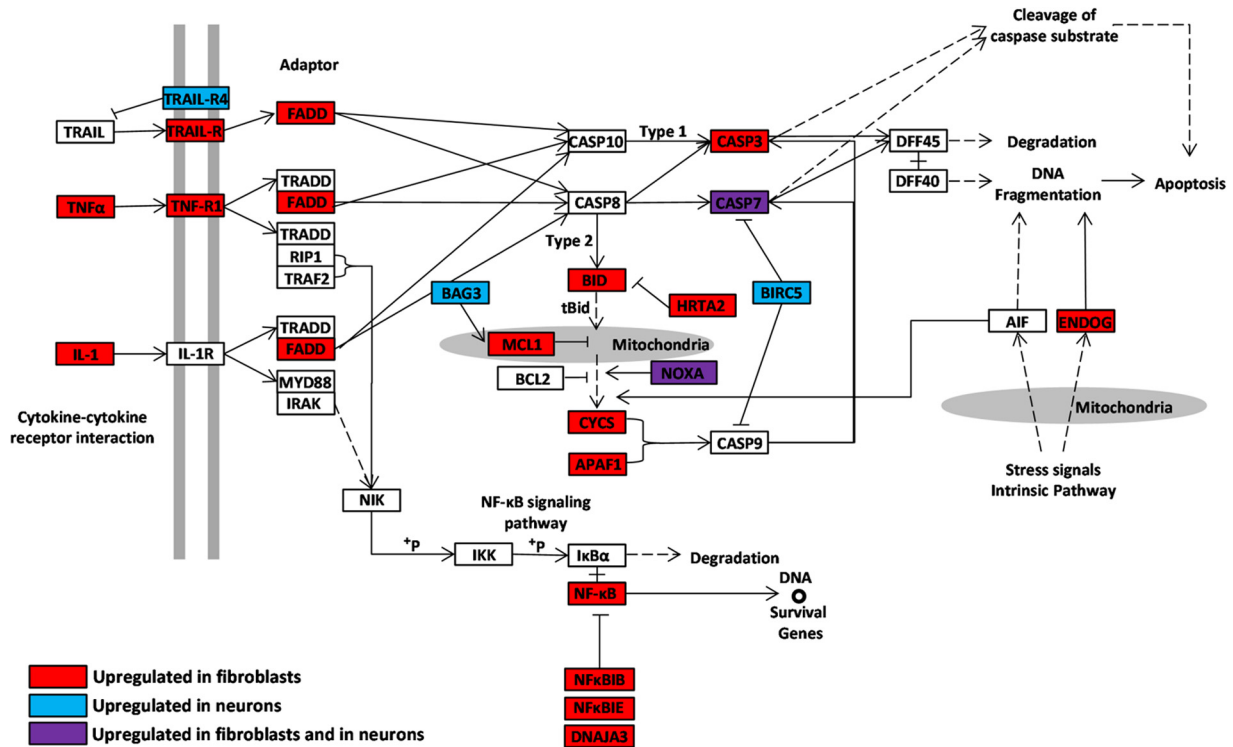


FIG 3 Distinct regulation of apoptosis pathway genes in neurons and fibroblasts in response to VZV infection. Pathway diagram modified from KEGG pathway database ([www.genome.jp/kegg/](http://www.genome.jp/kegg/)). Red boxes, genes upregulated in fibroblasts; blue boxes, those upregulated in neurons; purple boxes, upregulated genes common to the two cell types.

ificantly increased in fibroblasts and those in only one category were downregulated. Transcriptional changes in neurons were in 9 functional categories that differed strikingly from those of fibroblasts. Importantly, neurons did not show the fibroblast-specific upregulation of groups classified with the innate immune responses, NF- $\kappa$ B-related genes, and response to stress and DNA repair clusters.

The most striking difference in transcriptional changes elicited by VZV infection between neurons and HFF was that of apoptosis-related genes (Fig. 3). In fibroblasts, a large cluster of 193 proapoptotic genes was upregulated by infection. Transcripts for both ligands and receptors involved in apoptotic pathways were elevated (i.e., tumor necrosis factor alpha [TNF- $\alpha$ ], TNFR1, TRAIL-R, and interleukin-1 [IL-1]). In addition, some transcripts for genes downstream in the apoptosis pathway were elevated, including caspase-3, which plays a central role in execution of apoptosis. Several transcripts related to apoptosis were elevated (i.e., FADD, BID, CYCS, APAF-1, and ENDOG). Transcripts for the survival gene NF- $\kappa$ B subunit RelA were upregulated by VZV infection in fibroblasts, as well as transcripts for several NF- $\kappa$ B inhibitors (NF- $\kappa$ BIB, NF- $\kappa$ BIE, and DNAJA3). The NF- $\kappa$ B pathway in VZV-infected epidermal cells in SCID-Hu mice and in cultured fibroblasts has been examined in detail (2, 3). The interaction of VZV with apoptosis pathways in nonneurological cells has been the target of several investigations focusing on the cellular signaling pathways involved, especially those relating to phosphatidylinositol 3-kinase (PI3K) and AKT (9, 10; also reviewed in reference 11).

In contrast to the transcriptional inductions of apoptosis-related genes in fibroblasts that we observed, no clusters of proapoptotic

genes were upregulated in VZV-infected neurons. Instead, a cluster of 17 genes involved in antiapoptotic pathways was upregulated. Antiapoptotic genes upregulated in VZV-infected neurons include Trail-R4, BIRC5 (SURVIVIN), and BAG3. These changes reflect the observations that neurons productively infected with VZV do not undergo apoptosis *in vitro* (12, 13), as opposed to VZV-infected nonneurological cells. The antiapoptotic effect of VZV infections on neurons is similar to changes seen in neurons latently or productively infected by herpes simplex virus 1 (HSV-1) (reviewed in reference 8). Increased transcription of BIRC5 was observed in VZV-infected embryonic lung fibroblasts (14); however, that study was performed at early stages of infection (24 h) when cells were not yet undergoing apoptosis. Transcripts for a few genes, such as CASP7 and NOXA, were elevated in response to VZV infection in both neurons and fibroblasts.

In addition to the differences in transcriptional regulation relating to apoptosis, striking differences were observed in several GO categories between fibroblasts and neurons. Transcripts related to the NF- $\kappa$ B pathway, PML bodies, and the immune response were upregulated in fibroblasts but not neurons. The lack of upregulation of immune response genes is intriguing, in light of our recent observation that VZV (and HSV)-infected human neurons are less able to exclude superinfecting virus (15). Upregulation of transcripts in the development and WNT pathways was observed for neurons but not for fibroblasts.

In summary, the transcriptome pattern for VZV-infected neurons is strikingly different from that reported for fibroblasts, T cells, and VZV-infected keratinocytes (1, 4). The transcription changes in neurons productively infected with VZV described here likely reflect changes following primary infection or reactiva-

tion, rather than the latent state normally established in varicella disease. The activation of antiapoptotic pathways in neurons infected with VZV is therefore consistent with what would be predicted to occur *in vivo*. Future development of a consistent model for VZV latency using hESC-derived neurons should allow evaluation of the transcriptome of neurons in this state and comparison to that observed for other alphaherpesviruses (8).

**Nucleotide sequence accession number.** The raw data were deposited online in GEO (accession number [GSE54385](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE54385)).

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#### ADDENDUM IN PROOF

Relevant to this article is a report by N. L. Baird et al. (*J. Virol.* 88:5877–5880, 2014, doi:10.1128/JVI.00476-14) in which the VZV (not the cellular) transcriptome was compared between infected fibroblasts and neurons. Differential expression of only 12 VZV genes was observed, and the authors concluded that the lack of cell death in neurons is likely not to be a consequence of differential transcription of viral genes.

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