Gene expression changes in human eputhelial cells induced by a replication deficient human adenovirus type 26 vector in the context of using αvβ3 integrin as a receptor

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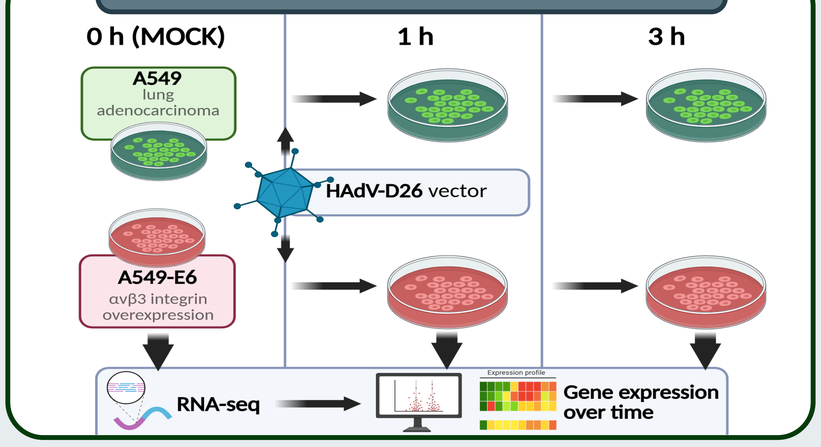
INTRODUCTION AND AIM

Human adenoviruses (HAdVs) are non-enveloped double stranded DNA viruses spanning more than 100 types classified into subgroups A-G. Due to its low seroprevalence and versatility as a gene delivery platform, human adenovirus type 26 (HAdV-D26) is an attractive candidate for vector design and gene therapy applications. Recently, two HAdV-D26-based vaccines were approved for use in humans for preventive purposes: a vaccine against Ebola and a vaccine against COVID-19. Despite their increasing clinical relevance, the exact mechanisms of HAdV-D26 infection and virus–host interactions still remain incompletely understood. Our previous work demonstrated that efficient cell entry and transduction of HAdV-D26 in epithelial cells strongly depends on the expression of αvβ3 integrin, suggesting that this receptor may play an important role in determining the early cellular response to infection. Here, we investigated the transcriptional response of human epithelial cells to HAdV-D26 infection in the context of αvβ3 integrin-mediated viral entry. RNA-seq was performed in A549 cells and A549-E6 cells overexpressing αvβ3 integrin at 1 and 3 h post-infection with an HAdV-D26 ΔE1/E3 (HAdV-D26 vector). We identified the differences in innate inflammatory and immunoregulatory responses, as well as cell adhesion and extracellular matrix regulation between the two cell lines, providing insights into the role of αvβ3 integrin in the early host response to HAdV-D26 infection.

MATERIALS AND METHODS



RESULTS

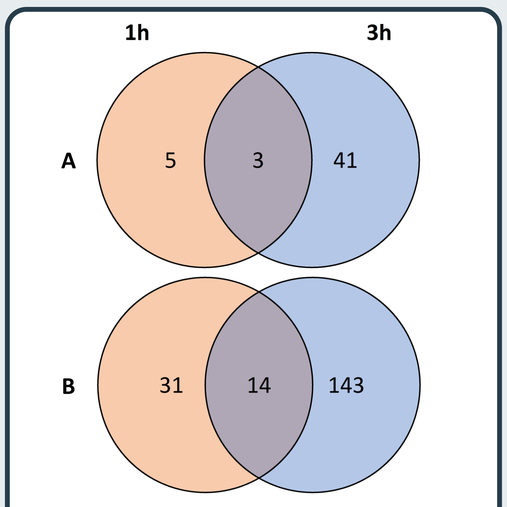


Figure 1. Number of significant differentially expressed genes (DEGs; adjusted P < 0.05) identified by RNA-seq at 1 and 3 h post-infection (hpi) in A549 (**A**) and A549-E6 (**B**) cells relative to mock-infected controls.

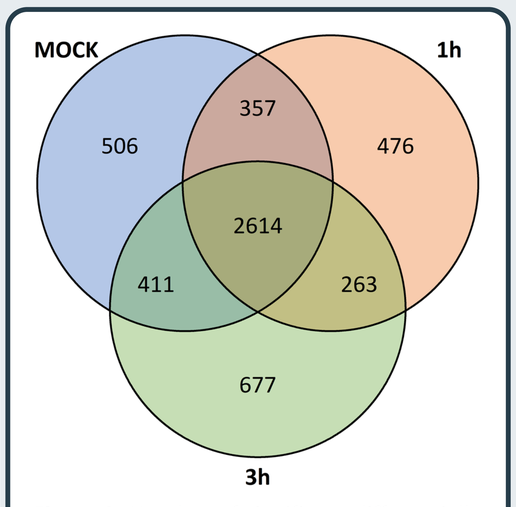


Figure 2. Number of significant differentially expressed genes (DEGs; adjusted P < 0.05) identified by RNA-seq in A549-E6 cells relative to A549 cells at mock-infected conditions, and at 1 and 3 hpi.

Table 1. Top 100 out of 172 total significant DEGs (|log₂FC|>1; filtered to include only infection-related named genes) ordered by ascending adjusted P-value

Gene symbol	Description	log₂FC		Gene symbol	Description	log₂FC	
		1h	3h			1h	3h
TRIM39-RPP21	TRIM39-RPP21 readthrough		31.4441	***RTL1***	retrotransposon Gag like 1	-4.3172	
KCNV1	potassium voltage-gated channel modifier V member 1	-8.2141		***DPEP1***	dipeptidase 1		-1.1375
NAP1L3	nucleosome assembly protein 1 like 3		2.8185	***TCHH***	trichohyalin	-1.2805	
TRPS1	transcriptional repressor GATA binding 1		-7.6083	***SFRP5***	secreted frizzled related protein 5	-1.2073	
ARHGEF6	Rac/Cdc42 guanine nucleotide exchange factor 6	3.1787	2.6506	***FSTL5***	follistatin like 5	-4.2448	
ISY1-RAB43	ISY1-RAB43 readthrough		28.0787	***IL12A***	interleukin 12A		1.1384
STX16-NPEPL1	STX16-NPEPL1 readthrough	7.7401		***TNFSF12-TNFSF13***	TNFSF12-TNFSF13 readthrough	4.7592	
SLC16A10	solute carrier family 16 member 10		-1.1596	***IL1B***	interleukin 1 beta	3.0395	2.9467
PSAPL1	prosaposin like 1	-1.4525		***DIRAS1***	DIRAS family GTPase 1		1.8357
NPNT	nephronectin	-2.6925		***GCHFR***	GTP cyclohydrolase I feedback regulator		-2.1784
LRRN3	leucine rich repeat neuronal 3	-2.2493	-3.7899	***SCEL***	sciellin	-4.2191	
TAPBPL	TAP binding protein like		-1.1129	***ARHGEF35***	Rho guanine nucleotide exchange factor 35	-2.7517	-1.8874
CYP1B1	cytochrome P450 family 1 subfamily B member 1		1.3351	***UGT2B7***	UDP glucuronosyltransferase family 2 member B7		-1.8122
BLOC1S5-TXNDC5	BLOC1S5-TXNDC5 readthrough		23.6455	***ANO9***	anoctamin 9	-1.3886	
PLA2G7	phospholipase A2 group VII	-6.0266	-5.4317	***NLRP3***	NLR family pyrin domain containing 3	3.6143	
IPO4	importin 4	-1.3649		***TOMM6***	translocase of outer mitochondrial membrane 6		-2.9114
RPS6KL1	ribosomal protein S6 kinase like 1	-2.6531		***SLC5A10***	solute carrier family 5 member 10	1.1434	
ATP1A2	ATPase Na+/K+ transporting subunit alpha 2	1.3432	2.1965	***GLIS3***	GLIS family zinc finger 3		-1.0737
SENP3-EIF4A1	SENP3-EIF4A1 readthrough		-3.2567	***HLA-DMB***	major histocompatibility complex, class II, DM beta	-1.1341	
SLC14A1	solute carrier family 14 member 1	-3.1342	-2.1541	***ADM2***	adrenomedullin 2	1.4229	
PLEKHA4	pleckstrin homology domain containing A4		2.0948	***CDH5***	cadherin 5	-1.3225	-3.1257
ZNF83	zinc finger protein 83	-1.73	-5.5226	***ADORA2A***	adenosine A2a receptor	3.2481	
UNC5D	unc-5 netrin receptor D	-1.5552		***CAMKV***	CaM kinase like vesicle associated	-1.5703	-1.7765
CDH19	cadherin 19		-4.7677	***CDH16***	cadherin 16		-3.2351
FAAH	fatty acid amide hydrolase	-1.4409	-1.1252	***OMG***	oligodendrocyte myelin glycoprotein	1.1681	1.0828
GAL	galanin and GMAP prepropeptide		-1.4418	***TC2N***	tandem C2 domains, nuclear		-3.1679
ATP6V1G2-DDX39B	ATP6V1G2-DDX39B readthrough		19.6711	***KLRG2***	killer cell lectin like receptor G2	-1.4727	
KLF5	KLF transcription factor 5		-1.0788	***PCDHGA5***	protocadherin gamma subfamily A, 5	1.9204	
MILR1	mast cell immunoglobulin like receptor 1	1.4882		***CLIC3***	chloride intracellular channel 3	1.6258	
NCAM2	neural cell adhesion molecule 2		-5.3586	***TMEM74***	transmembrane protein 74	-1.798	
ACP7	acid phosphatase 7, tartrate resistant	-5.4592		***MYO15A***	myosin XVA		2.1713
EFCAB5	EF-hand calcium binding domain 5	1.5354		***TUBAL3***	tubulin alpha like 3	2.0218	
IRF4	interferon regulatory factor 4		2.1758	***CYP4F3***	cytochrome P450 family 4 subfamily F member 3	-2.1576	
HK2	hexokinase 2		1.9788	***ZNF846***	zinc finger protein 846		1.1465
SLC23A3	solute carrier family 23 member 3	1.6857		***CD300C***	CD300c molecule		2.2631
MALRD1	MAM and LDL receptor class A domain containing 1	2.4013	3.6848	***VTCN1***	V-set domain containing T cell activation inhibitor 1	-2.8386	
FAAH2	fatty acid amide hydrolase 2		-1.2857	***RSPH14***	radial spoke head 14 homolog	-2.1058	-1.9776
OPRL1	opioid related nociceptin receptor 1		1.4081	***PCBP3***	poly(rC) binding protein 3		2.3511
VCAM1	vascular cell adhesion molecule 1		4.4521	***IQCN***	IQ motif containing N	-1.7908	
HDAC9	histone deacetylase 9		1.2555	***HTR1B***	5-hydroxytryptamine receptor 1B	1.7492	
RAB7B	RAB7B, member RAS oncogene family		2.4368	***PRSS22***	serine protease 22	-2.2228	
PPP1R16B	protein phosphatase 1 regulatory subunit 16B	-1.8412	-1.9172	***COL5A3***	collagen type V alpha 3 chain		2.3715
NAB2	NGFI-A binding protein 2	1.0429		***PTPRD***	protein tyrosine phosphatase receptor type D		-2.532
NRXN3	neurexin 3		-3.9133	***LDB3***	LIM domain binding 3		1.181
NR1H4	nuclear receptor subfamily 1 group H member 4	-1.5492	-3.0564	***LRRC19***	leucine rich repeat containing 19		-1.8643
MED12L	mediator complex subunit 12L		-1.4774	***UGT1A6***	UDP glucuronosyltransferase family 1 member A6		-1.7381
ACTL8	actin like 8	-1.5502		***ZNF20***	zinc finger protein 20	1.6985	
STON2	stonin 2	1.1134		***GPIHBP1***	high density lipoprotein binding protein 1	2.4222	
PCSK1	proprotein convertase subtilisin/kexin type 1		2.3889	***ECT2L***	epithelial cell transforming 2 like	-1.7907	
TMEM215	transmembrane protein 215		2.4265	***NUDT10***	nudix hydrolase 10		1.7668

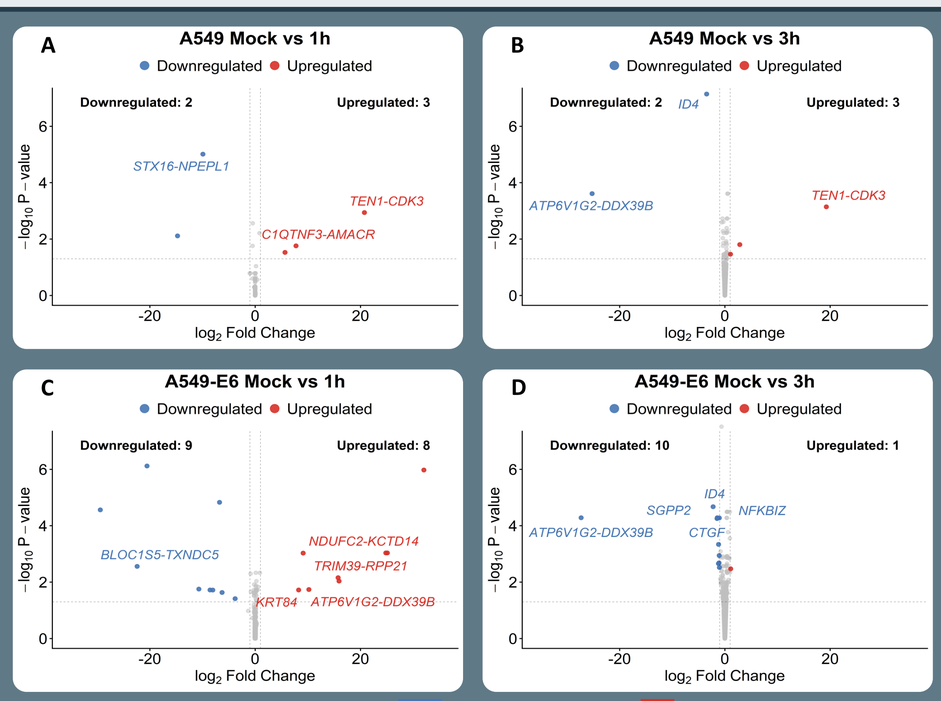


Figure 3. Significant downregulated (blue) and upregulated (red) differentially expressed genes (DEGs; adjusted P < 0.05; |log₂FC| > 1) identified by RNA-seq in A549 at 1 h (**A**), and 3h (**B**) post-infection relative to A549 mock-infected cells, and in A549-E6 cells at 1 h (**C**), and 3h (**D**) post-infection relative to A549-E6 mock-infected cells. Top 5 significant named genes by adjusted P-value are labelled.

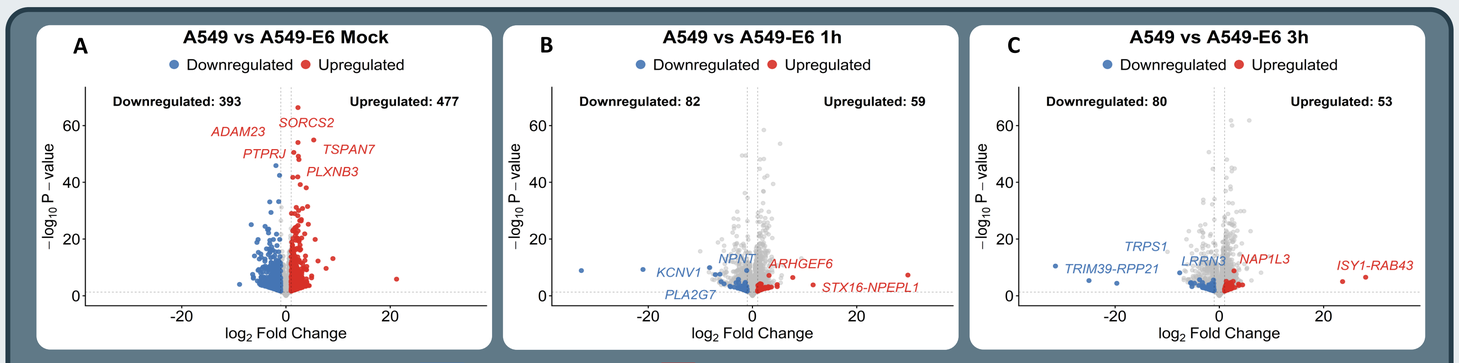


Figure 4. Significant downregulated (blue) and upregulated (red) differentially expressed genes (DEGs; adjusted P < 0.05; |log₂FC| > 1) identified by RNA-seq in A549-E6 cells relative to A549 cells under mock-infected conditions (**A**), and at 1 h (**B**) and 3 h (**C**) post-infection (hpi). For the 1 and 3 hpi comparisons, DEGs that were also detected under mock-infected conditions were excluded unless they met the |log₂FC| > 1 threshold. This filtering removed genes associated with baseline differences between the cell lines, leaving only DEGs specifically associated with HAdV-D26 infection.

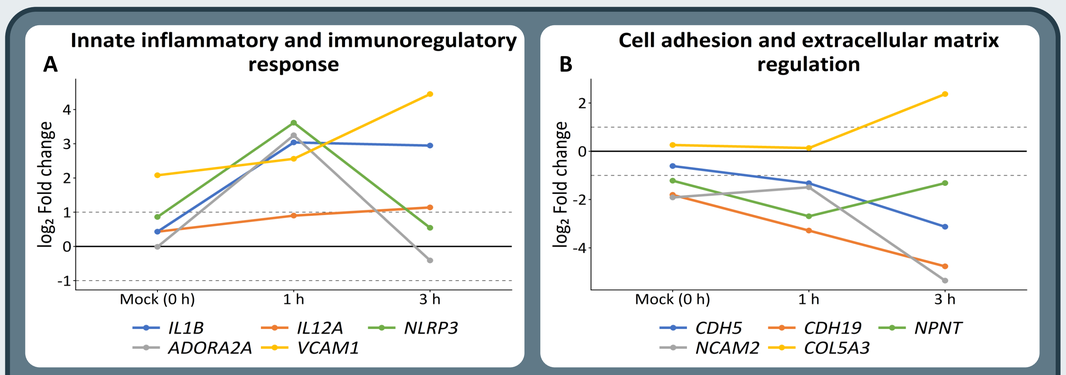


Figure 5. Change in expression over time in A549-E6 cells relative to A549 cells shown for selected DEGs associated with cellular responses to infection. (**A**) Selected genes connected with innate inflammatory and immunoregulatory responses, showing increased expression during the early stages of infection. *NLRP3* and *ADORA2A* show upregulation at 1 hpi before returning to base expression levels, while *IL1B*, *IL12A*, and *VCAM1* are consistently upregulated during early infection, indicating activation of inflammatory and immunoregulatory pathways. (**B**) Selected genes associated with cellular adhesion and extracellular matrix interactions, showing mixed transcriptional regulation over time. *CDH5*, *CDH19*, *NPNT* and *NCAM2* are downregulated, while *COL5A3* is upregulated at 3 h. These mixed transcriptional changes suggest altered cellular adhesion and cellular matrix interactions in early infection time points as a result of αvβ3 integrin-mediated infection.

CONCLUSIONS

- The early transcriptional response of human epithelial cells to HAdV-D26 infection is different depending on αvβ3 integrin expression levels. We identified **172 genes** associated with early HAdV-D26 infection that are significantly differentially expressed between A549 and A549-E6 cells at 1 and 3 h post-infection.
- Several of these genes are involved in **innate inflammatory and immunoregulatory responses**, including *IL1B, IL12A, NLRP3, ADORA2A,* and *VCAM1*, as well as **cell adhesion and extracellular matrix regulation**, including *CDH5, CDH19, NPNT, NCAM2,* and *COL5A3*.
- These findings suggest that **αvβ3 integrin abundance may influence the early host epithelial cell response to HAdV-D26 infection**, potentially contributing to differences in virus–host interactions during the early stages of infection.

Scan for more data!

Institut Ruđer Bošković
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