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Elucidating the host–pathogen interaction between human colorectal cells and invading Enterovirus 71 using transcriptomics profiling

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ABSTRACT

Enterovirus 71 (EV71) is one of the main etiological agents for Hand, Foot and Mouth Disease (HFMD) and has been shown to be associated with severe clinical manifestation. Currently, there is no antiviral therapeutic for the treatment of HFMD patients owing to a lack of understanding of EV71 pathogenesis. This study seeks to elucidate the transcriptomic changes that result from EV71 infection. Human whole genome microarray was employed to monitor changes in genomic profiles between infected and uninfected cells. The results reveal altered expression of human genes involved in critical pathways including the immune response and the stress response. Together, data from this study provide valuable insights into the host–pathogen interaction between human colorectal cells and EV71.

1. Introduction

Enterovirus 71 (EV71) is a positive sense, single stranded, non-enveloped RNA virus belonging to Enterovirus genus of the Picornaviridae family [1–4]. It is one of the main etiological agents for Hand, Foot and Mouth Disease (HFMD) which commonly affects infants and children. This disease is usually self-limiting, characterised by various symptoms such as fever, rashes, poor appetite and multiple ulcers in mouth [5–7]. The route of transmission of EV71 was postulated to happen *via* direct contact of vesicular fluid or droplet from the infected or *via* faecal-oral route [5,8–10]. EV71 was shown to replicate within the gastrointestinal tract, bypass the gut barrier and infect into the skeletal muscle cell before entering into the bloodstream and the central nervous system [5,8–10]. However, unlike other HFMD causing enteroviruses, EV71 have been commonly associated with severe clinical diseases, including neurological diseases leading to cardiopulmonary failure and death.

EV71 was first described in 1974 in CA (USA) after it was isolated from patients with central nervous system disease in 1969 [11]. Recent molecular evolution studies have predicted that EV71 could have emerged in the human population as early as 1941 [12–14]. Large fatal outbreaks of HFMD first appeared in Bulgaria in 1975, and disease outbreaks were subsequently identified in Hungary in 1978 and re-emerged after two decades in Malaysia in 1997 and Taiwan in 1998 [15–19]. Since then, there have been various outbreaks, epidemics and pandemics that have periodically been reported worldwide with outbreaks occurring every two to three years in countries including Australia, China, Taiwan, Japan, Korea, Malaysia, Vietnam, Thailand and Singapore [7,11,15,20].

Currently and due in part to a lack of understanding of viral pathogenesis of EV71 causing HFMD, there are no effective antiviral therapies or vaccines approved by the United States Food and

Abbreviations: EV71, Enterovirus 71; HFMD, Hand, Foot and Mouth Disease; ISG, interferon stimulated genes; MOI, multiplicity of infection; RD, rhabdomyosarcoma

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Drug Administration (FDA) to prevent HFMD. There is therefore a need to gain a better understanding of the mechanism of EV71 pathogenesis. The ability to cause infection directly correlates to the interaction between the host and pathogen. In order to survive and proliferate during infection, the pathogen must be able to sense, react and adapt to the ever-changing microenvironment within the host. Such adaptations and the use of the host resources to proliferate are prerequisites for the successful colonisation of the host and establish disease. Viruses, being obligate intracellular parasites have developed highly sophisticated mechanisms not only to exploit the biosynthetic machinery for replication, but also to specifically evade the host immune system.

During infection, viruses regulate host gene expression to enhance their survival. Such activities include altering the cellular microenvironment to allow successful virus replication and evasion of the host immune system [21–23]. Elucidating the mechanism that the virus uses to bypass host defence systems and establish infections will provide us with a better understanding of the pathogenesis mechanism of the EV71 virus and will thus aid in the development of potential antiviral therapeutics for HFMD patients. In this study, transcriptomics profiling was performed using colorectal cells infected with EV71 using mRNA microarray.

2. Materials and methods

2.1. Cell culture and virus propagation

Human colorectal cell line (HT29) (ATCC® Catalog No. HTB-38™) was maintained in Roswell Park Memorial Institute medium (RPMI) (PAA Laboratories, Austria) supplemented with 10% (v/v) Fetal Bovine Serum (FBS) (PAA Laboratories, Austria) and 2% penicillin–streptomycin (PAA Laboratories, Austria) at 37 °C with 5% CO_2. The EV71 strain used in this study was isolated from a fatal case of HFMD during October 2000 outbreak in Singapore, Enterovirus 5865/sin/000009 strain from subgenogroup B4 (accession number 316321; hereby designated as Strain 41). The virus stock was prepared by propagation of viruses using 90% confluent HT29 cells monolayer in RPMI with 10% FBS at 37 °C with 5% CO_2. The virus titres were determined using 50% tissue culture infective dose ($TCID_{50}$) per millilitre (mL) according to Reed and Muench method [24].

2.2. EV71 infection for mRNA profiling

HT29 cells were seeded at a concentration of 5×10^5 cells/mL in each well of a 6-well plate and incubated for 24 h at 37 °C with 5% CO_2. Cells were washed twice with phosphate buffered saline (PBS) and infected with EV71 at multiplicity of infection (MOI) of 1 or nil respectively. The culture media were removed and replaced with 2 mL of fresh RPMI medium after 1 h of incubation at 37 °C with 5% CO_2. The respective infected cells were harvested at 36 h posts infection (hpi). The total cellular RNA of HT29 cells were extracted using the miRNeasy mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Briefly, the cells were lysed and homogenise using lyses solution provided (Qiagen, Hilden, Germany). Total RNA were purified using the RNeasy spin column and eluted (Qiagen, Hilden, Germany). Harvested total RNA was quantitated using Nanodrop 100 spectrophotometer (ThermoScientific, Waltham, USA).

2.3. mRNA microarray analysis

Extracted total RNA were labelled with GeneChip HT 3′ IVT Express Kit following manufacturer's instruction. Hybridization to GeneChip PrimeView Human Gene Expression Array was performed in accordance to manufacturer's recommendations. Every chip was scanned at a high resolution by the Affymetrix GeneChip Scanner 3000 according to the GeneChip Expression Analysis Technical Manual procedures (Affymetrix, Santa Clara, CA). Briefly, total extracted RNA undergo two rounds of cDNA synthesis prior to *in vitro* transcription to synthesise biotin modified amplified RNA (Affymetrix, Santa Clara, CA). The amplified RNA was purified and fragment for the hybridisation onto the GeneChip PrimeView Human Gene Expression Array (Affymetrix, Santa Clara, CA). Raw data for the microarray were analysed with the Partek® Genomics Suite (Partek Incorporated, Saint Louis, USA) where $p < 0.05$ is considered significant.

2.4. cDNA synthesis and quantitative real time polymerase chain reaction

For the conversion of mRNA to cDNA, 1 μg of the total RNA was reverse transcripted using using the iScript™ cDNA Synthesis Kit (Bio-Rad Laboratories, CA, USA) in accordance to the manufacturer's instructions. Briefly, 1 μg of the extracted RNA was mixed with enzyme reverse transcriptase and buffer to a volume of 20 μl and subjected to thermal profile of 25 °C for 5 min, 42 °C for 30 min followed by 85 °C for 5 min in accordance to the manufacturer's instructions.

The quantitative real time polymerase chain reaction (qPCR) was performed using the iTaq™ Universal SYBR® Green Supermix (Bio-Rad Laboratories, CA, USA) on the BioRad CFX96™ Real-Time PCR system (Bio-Rad Laboratories, CA, USA). The primers used in this study are listed in Table 1. Briefly, 1 μl of cDNA and 1 μl of the forward and the reverse primers were added to iTaq™ Universal SYBR® Green Supermix. The reaction mix was then subjected to thermal profile of denaturation at 95 °C for 30 s, followed by amplification and quantification in 40 cycles at 95 °C for 5 s followed by 60 °C for 30 s. At the end of amplification cycles, melting temperature analysis was performed by the BioRad CFX96TM Real-Time PCR system (Bio-Rad Laboratories, CA, USA). Relative gene expression was quantified based on $2^{-\Delta\Delta CT}$ method using the housekeeping gene β-actin as the reference gene [25].

2.5. Data analysis

All statistical analysis was performed on GraphPad Prism Version 6.0c (GraphPad Software, USA). Student's *t* test was used to compare two groups. *p* values of <0.05 were considered statistically significant.

3. Result and discussion

Systemic analyses of host response to EV71 provide critical clues to understand the molecular mechanism of EV71 pathogenesis

Table 1
Primers used in this study.

Primers	5′–3′
EV71-VP1-reverse	GCTCTATAGGAGATAGTGTGAGTAGGG
EV71-VP1-forward	ATGACTGCTCACCTGCGTGTT
IL29/IFN lambda1-forward	ACCGTGGTGCTGGTGACTT
IL29/IFN lambda1-reverse	CTAGCTCCTGTGGTGACAGA
IFN beta1-forward	ATGACCAACAAGTGTCTCCTCC
IFN beta1-reverse	GGAATCCAAGCAAGTTGTAGCTC
IFN gamma1-forward	TCTTTGGGTCAGAGTTAAAGCCA
IFN gamma1-reverse	TTCCATCTCGGCATACAGCAA
ISG54-forward	AAGCACCTCAAAGGGCAAAAC
ISG54-reverse	TCGGCCCATGTGATAGTAGAC
ISG56-forward	TTGATGACGATGAAATGCCTGA
ISG56-reverse	CAGGTCACCAGACTCCTCAC
β-Actin forward	ACCAACTGGGACGACATGGAGAAA
β-Actin reverse	TAGCACAGCCTGGATAGCAACGTA

during infection of a human host. Regulation and alteration of the host cellular system can also be monitored by studying the host cellular transcriptomics change following enterovirus infection. Transcriptomics analysis *via* RNA-sequencing, mRNA microarray and PCR array have been previously used to analyse host gene expression [26–28]. However, these studies have been performed on rhabdomyosarcoma (RD) cells or SH-SY5Y cells, of muscle cells or neuronal origin respectively. In a clinical context, this may not be a good representative model during EV71 infection of a human host as various studies have demonstrated that the gastrointestinal tract was the first site for EV71 proliferation [29–31]. Considering that different cell types have varying cellular content, host–pathogen interaction may differ from cell types to cell types [1,32,33]. We have previously reported a human colorectal adenocarcinoma cell line (HT29) with epithelioid morphology as a useful *in vitro* model to study the pathogenesis of EV71 [33]. To this end, this study

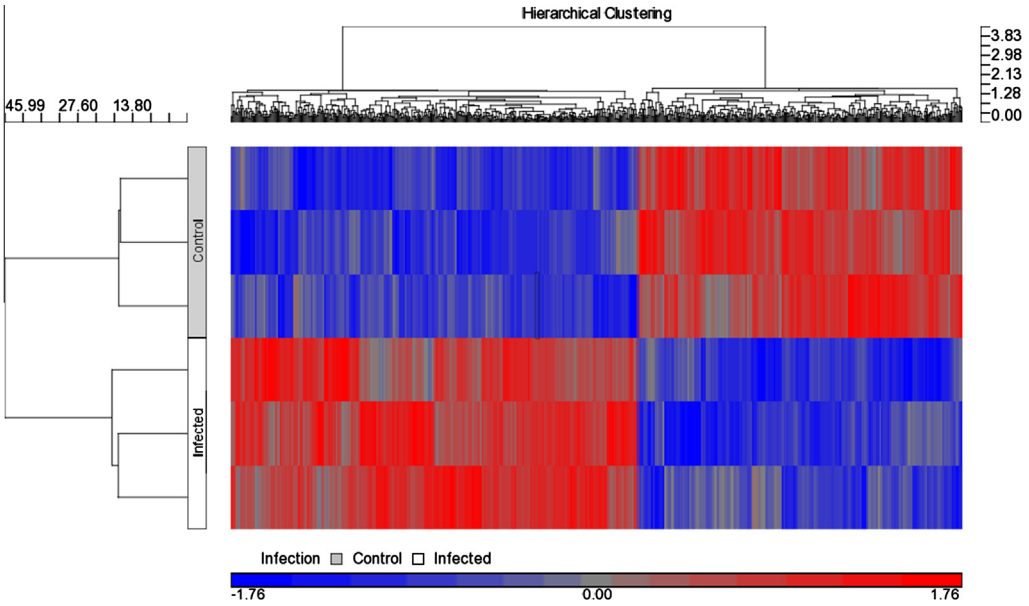

Fig. 1. Heat map of the microarray mRNA expression profile of EV71 infected and non-infected control colorectal cells, HT29. The two-way hierarchical cluster heat map showed differential expressed mRNAs of two groups of samples. The mRNAs were chosen according to the cut off $p < 0.05$ where blue represents mRNAs with decreased expression and red represent mRNAs with increased expression. ($n = 3$, $^{*}p$ values of <0.05).

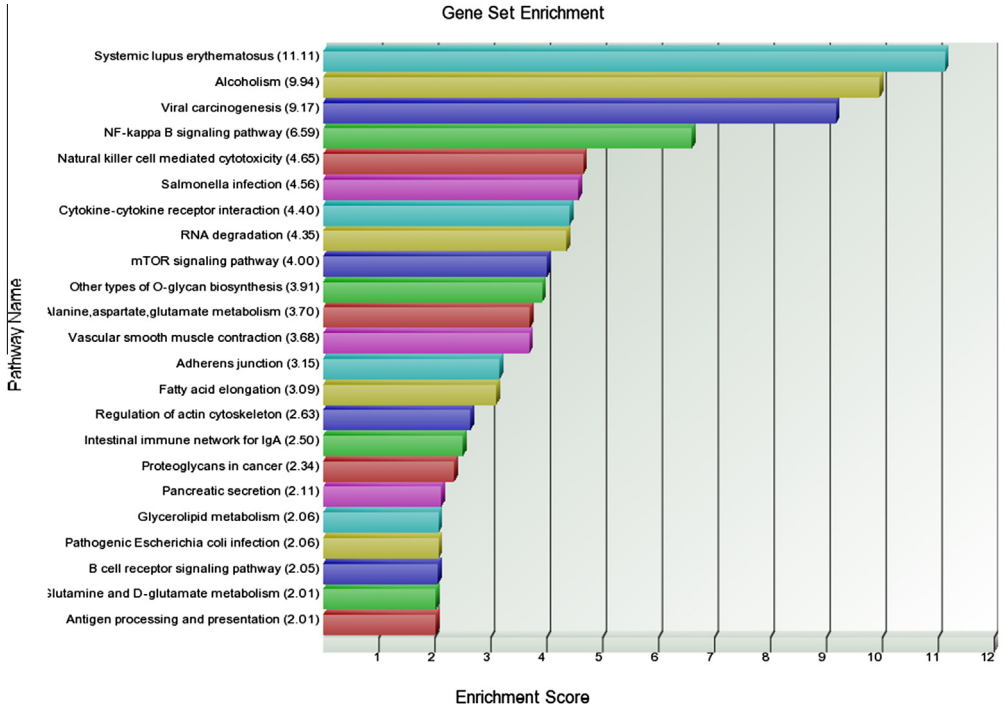

Fig. 2. Pathway analysis of differentially expressed genes of EV71 infected and non-infected control colorectal cells, HT29. Pathways with enrichment score of <2 was generated using Partek® Genomics Suite (Partek Incorporated, Saint Louis, USA) ($n = 3$).

utilised HT29 as a model for the transcriptomics analysis of host response to EV71 infection.

3.1. Identification of deregulated mRNAs during EV71 infection

To identify the differentially transcribed mRNAs in HT29 during EV71 infection, we perform mRNAs profiling using GeneChip PrimeView Human Gene Expression Array. Comparative mRNAs expression between EV71 infected cells and control non-infected cells were performed. Microarray hybridisation identified 699 genes being differentially expressed significantly during EV71 infection ($p < 0.05$) (Fig. 1 and Supplementary Table 1). Using Partek® Genomics Suite, 699 differentially transcribed genes between EV71 infected and control non-infected HT29 were further analyses to generate pathways with enrichment score of <2 (Fig. 2). These differentially transcribed genes were involved in critical pathways including the immune response, the stress response, metabolism, cytotoxicity, cytokines and the cytoskeleton network (Fig. 2).

In response to viral infection, cells were known to induce an immunological response to combat against invading pathogens. In particular, immune response cytokines IL29/interferon (IFN)-λ1 and IFN-β1 were significantly up-regulated in EV71 infected cells (Supplementary Table 1). The microarray results were validated using qPCR. In line with the microarray findings, qPCR revealed that both IL29 and IFN-β1 were significantly up-regulated by approximately 20 fold and 3 fold in EV71 infected cells respectively (Fig. 3a and b). This is not surprising as that both IL29 and IFN-β1 are cytokines which play important roles in host antiviral response [34–36]. In addition, IL29 has been previously reported to be up regulated during viral infection such as influenza A, hepatitis C virus and Sendai viruses [34–36]. A key characteristic of cytokines is the ability to interact with one another to establish coordinated defence mechanism against invading pathogens. IL29 functions by binding to a unique receptor through a pathway identical to IFN-β1 and IFN-γ1 receptors inducing cellular antiviral activity to inhibit virus replication [34–36]. Synergetic effect between IL29, IFN-β1 and IFN-γ1 were shown to promote cellular

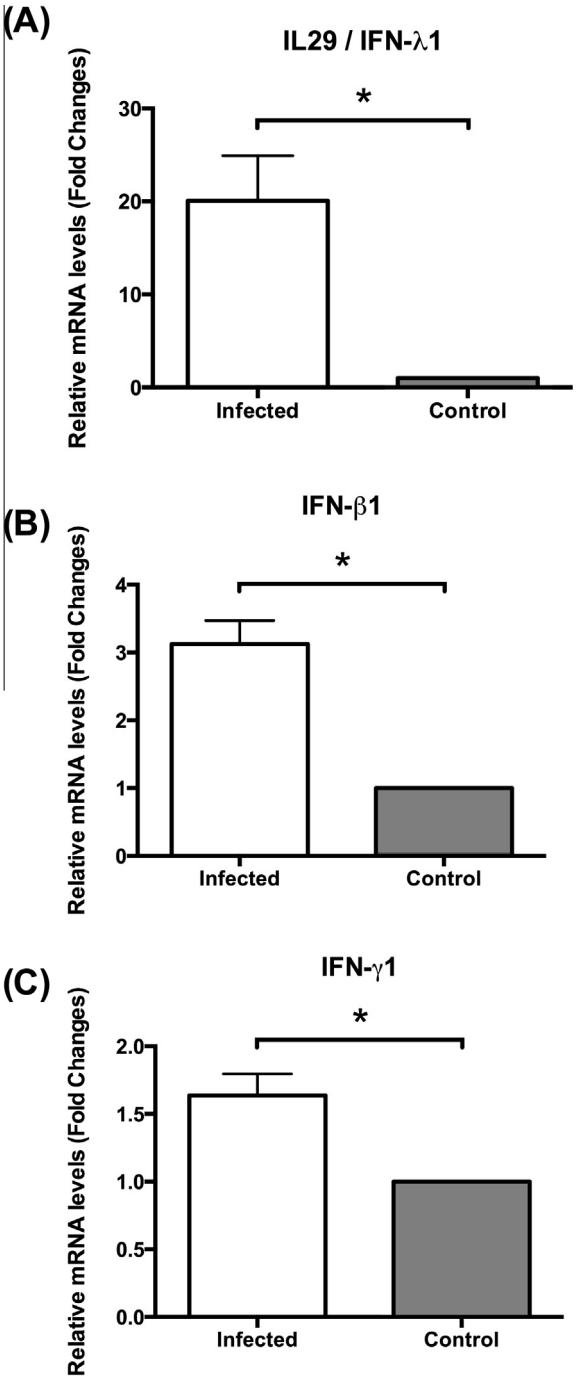

Fig. 3. Expression level of types I, II and III IFN signalling pathways in response to EV71 infection. Confluent HT29 cells were infected with or without EV71 (MOI of 1). Total intracellular RNA were harvested 36 hpi, converted to cDNA and measured by quantitative real time polymerase chain reaction (qPCR). (A) Expression of IL29/IFN-λ1; (B) expression of IFN-β1; (C) expression of IFN-γ1 ($n = 3$, $^*p$ values of <0.05).

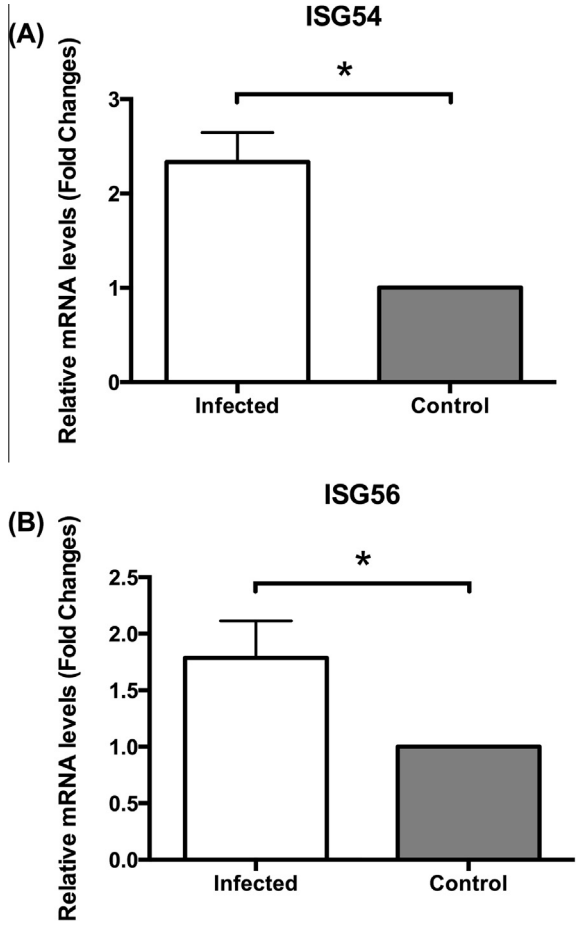

Fig. 4. Expression level of interferon stimulated genes in response to EV71 infection. Confluent HT29 cells were infected with or without EV71 (MOI of 1). Total intracellular RNA were harvested 36 hpi, converted to cDNA and measured by quantitative real time polymerase chain reaction (qPCR). (A) Expression of ISG54; (B) expression of ISG56 ($n = 3$, $^*p$ values of <0.05).

antiviral response [34–36]. Indeed, the use of IL29, IFN-β1 and IFN-γ1 as antiviral treatment has been shown to inhibit various viruses including herpes simplex-1 virus, mouse hepatitis virus type 2, human T-lymphotropic virus-1, cytomegalovirus and hepatitis C virus [37–43]. As such during EV71 infection, HT29 may up regulate cytokines such as IL29, IFN-β1 and IFN-γ1 (types I, II and III IFN) to induce interferon stimulated genes (ISG) as part of cellular antiviral response to slow down virus replication.

To further verify this reasoning, we performed qPCR on IFN-gamma1 receptor, ISG54 and ISG56 to investigate if these genes have been up regulated during EV71 infection. In agreement with our hypothesis, our qPCR results show significant up regulation of these cytokines in response to EV71 infection (Figs. 3c and 4a, b). This support notion that the host colorectal cells up-regulate cytokines such as IL29, IFN-β1 and IFN-γ1 (types I, II and III IFN) possibly by a series of JAK-STAT signalling pathways to induce ISG such as ISG54 and ISG56 (Fig. 5). However, Lei et al. (2010, 2011, 2013) previously reported that EV71 3C protein suppressed the host immune system, for example type I interferon, during infection in RD cells (muscle cells) [44–46]. As such, the mechanism whereby EV71 mediate host immune system may differ from cell type to cell types. To support this hypothesis, Chi et al. (2013) have compared the expression of IFN-signalling pathway between intestinal epithelial cells and muscle cells, which demonstrates that intestinal cells have indeed a significant higher expression of cytokines response in comparison with muscle cells [47]. This may explain the fact it takes three times longer for the virus to kill HT29 cells in comparison with RD cells [32,33]. In addition, IFN signalling pathways such as type I interferon was previously demonstrated to play an important role during EV71 pathogenesis [48].

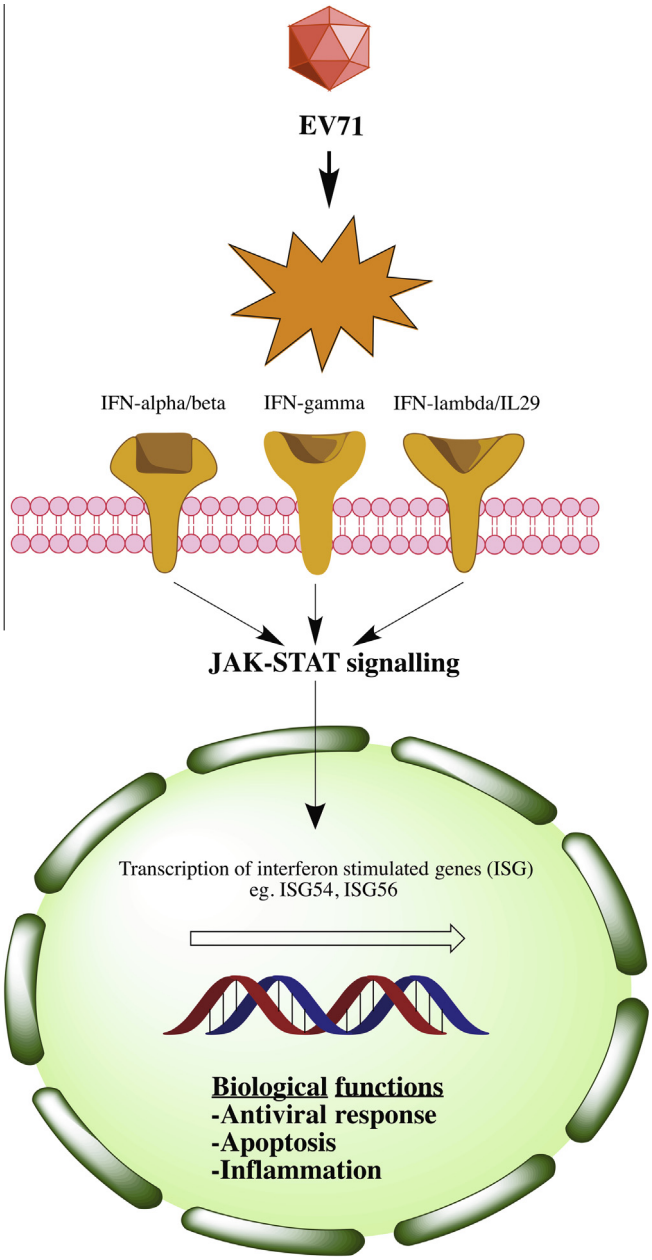

Fig. 5. Schematic illustration of the interplay between types I, II and III IFN pathways and invading viral pathogen-EV71. Following EV71 infection, host activate types I, II and III IFN pathways through a series of JAK-STAT signalling cascade to mobilise interferon stimulated genes such as ISG54 and ISG56 in attempt to slow down virus replication.

Effectively, the intestinal epithelial immune system, being the initial site of infection, plays a critical role in the disease progression and clinical prognosis. Impaired or immature immunity has previously been associated with increased morbidity and mortality in EV71 of young children and immune deficient adults [48].The ability to eliminate viral pathogen such as EV71 at the initial site of infection is critical as a weaker immune response results in a higher viral titre leading to a systemic spread with more serve complications [47]. One of the key antiviral responses that the intestinal epithelial immune system utilises may be the types I, II and III IFN to eliminate invading EV71 (Fig. 5).

4. Conclusion

In conclusion, this study utilised HT29 as an *in vitro* model to investigate on the transcriptomic changes in human colorectal cells in response to EV71 infection. Human whole genome microarray was employed to monitor changes in profiles between EV71 infected cells and uninfected control cells. The result reveals altered expression of human mRNAs in selected pathways including the immune response, the stress response, metabolism, cytotoxicity, cytokines and the cytoskeleton network. Interestingly, our results shows significant up regulation of types I, II and III IFN which suggests that during EV71 infection, this may be the key antiviral response HT29 cells undertake to eliminate invading EV71. Taken together, data from this study provide valuable fundamental information toward understanding of host–pathogen interaction between human colorectal cells and invading EV71. Elucidating the mechanism that the virus uses to bypass host defence systems and establish infections will provide us with a better understanding of the pathogenesis mechanism of the EV71 virus and will thus aid in the development of potential antiviral therapeutics for HFMD patients.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Y.L.E.L. perform the work, evaluate the result and wrote the paper. Y.L.E.L., T.L.T., L.M.H., P.T., K.H.T., E.L.T. provide research idea, design the work and evaluate manuscript. All authors read and approved the final manuscript.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fob.2014.04.005.

References

[1] Lui, Y.L.E., Lin, Z., Lee, J.J., Chow, V.T.K., Poh, C.L. and Tan, E.L. (2013) Beta-actin variant is necessary for enterovirus 71 replication. Biochem. Biophys. Res. Commun. 433, 607–610.

[2] McMinn, P.C. (2002) An overview of the evolution of enterovirus 71 and its clinical and public health significance. FEMS Microbiol. Rev. 26, 91–107.

[3] McMinn, P.C. (2012) Recent advances in the molecular epidemiology and control of human enterovirus 71 infection. Curr. Opin. Virol. 2, 199–205.

[4] Ooi, M.H., Wong, S.C., Lewthwaite, P., Cardosa, M.J. and Solomon, T. (2010) Clinical features, diagnosis, and management of enterovirus 71. Lancet Neurol. 9, 1097–1105.

[5] Wong, S.S., Yip, C.C., Lau, S.K. and Yuen, K.Y. (2010) Human enterovirus 71 and hand, foot and mouth disease. Epidemiol. Infect. 138, 1071–1089.

[6] Ho, B.C., Yu, S.L., Chen, J.J., Chang, S.Y., Yan, B.S., Hong, Q.S., Singh, S., Kao, C.L., Chen, H.Y., Su, K.Y., Li, K.C., Cheng, C.L., Cheng, H.W., Lee, J.Y., Lee, C.N. and Yang, P.C. (2011) Enterovirus-induced miR-141 contributes to shutoff of host protein translation by targeting the translation initiation factor eIF4E. Cell Host Microbes 9, 58–69.

[7] Huang, H.I., Weng, K.F. and Shih, S.R. (2012) Viral and host factors that contribute to pathogenicity of enterovirus 71. Future Microbiol. 7, 467–479.

[8] Lee, T.C., Guo, H.R., Su, H.J., Yang, Y.C., Chang, H.L. and Chen, K.T. (2009) Diseases caused by enterovirus 71 infection. Pediatr. Infect. Dis. J. 28, 904–910.

[9] Liu, C.C., Tseng, H.W., Wang, S.M., Wang, J.R. and Su, I.J. (2000) An outbreak of enterovirus 71 infection in Taiwan, 1998: epidemiologic and clinical manifestations. J. Clin. Virol. 17, 23–30.

[10] Brown, B.A., Oberste, M.S., Alexander Jr., J.P., Kennett, M.L. and Pallansch, M.A. (1999) Molecular epidemiology and evolution of enterovirus 71 strains isolated from 1970 to 1998. J. Virol. 73, 9969–9975.

[11] Schmidt, N.J., Lennette, E.H. and Ho, H.H. (1974) An apparently new enterovirus isolated from patients with disease of the central nervous system. J. Infect. Dis. 129, 304–309.

[12] Lee, M.S., Tseng, F.C., Wang, J.R., Chi, C.Y., Chong, P. and Su, I.J. (2012) Challenges to licensure of enterovirus 71 vaccines. PLoS Negl. Trop. Dis. 6, e1737.

[13] Tee, K.K., Lam, T.T., Chan, Y.F., Bible, J.M., Kamarulzaman, A., Tong, C.Y., Takebe, Y. and Pybus, O.G. (2010) Evolutionary genetics of human enterovirus 71: origin, population dynamics, natural selection, and seasonal periodicity of the VP1 gene. J. Virol. 84, 3339–3350.

[14] van der Sanden, S., Koopmans, M., Uslu, G. and van der Avoort, H. (2009) Epidemiology of enterovirus 71 in the Netherlands, 1963 to 2008. J. Clin. Microbiol. 47, 2826–2833.

[15] Chumakov, M., Voroshilova, M., Shindarov, L., Lavrova, I., Gracheva, L., Koroleva, G., Vasilenko, S., Brodvarova, I., Nikolova, M., Gyurova, S., Gacheva, M., Mitov, G., Ninov, N., Tsylka, E., Robinson, I., Frolova, M., Bashkirtsev, V., Martiyanova, L. and Rodin, V. (1979) Enterovirus 71 isolated from cases of epidemic poliomyelitis-like disease in Bulgaria. Arch. Virol. 60, 329–340.

[16] Nagy, G., Takatsy, S., Kukan, E., Mihaly, I. and Domok, I. (1982) Virological diagnosis of enterovirus type 71 infections: experiences gained during an epidemic of acute CNS diseases in Hungary in 1978. Arch. Virol. 71, 217–227.

[17] Ho, M., Chen, E.R., Hsu, K.H., Twu, S.J., Chen, K.T., Tsai, S.F., Wang, J.R. and Shih, S.R. (1999) An epidemic of enterovirus 71 infection in Taiwan. Taiwan Enterovirus Epidemic Working Group. N. Engl. J. Med. 341, 929–935.

[18] Lu, C.Y., Lee, C.Y., Kao, C.L., Shao, W.Y., Lee, P.I., Twu, S.J., Yeh, C.C., Lin, S.C., Shih, W.Y., Wu, S.I. and Huang, L.M. (2002) Incidence and case-fatality rates resulting from the 1998 enterovirus 71 outbreak in Taiwan. J. Med. Virol. 67, 217–223.

[19] Solomon, T., Lewthwaite, P., Perera, D., Cardosa, M.J., McMinn, P. and Ooi, M.H. (2010) Virology, epidemiology, pathogenesis, and control of enterovirus 71. Lancet Infect. Dis. 10, 778–790.

[20] McMinn, P., Stratov, I., Nagarajan, L. and Davis, S. (2001) Neurological manifestations of enterovirus 71 infection in children during an outbreak of hand, foot, and mouth disease in Western Australia. Clin. Infect. Dis. 32, 236–242.

[21] Scaria, V., Hariharan, M., Maiti, S., Pillai, B. and Brahmachari, S.K. (2006) Host–virus interaction: a new role for microRNAs. Retrovirology 3, 68.

[22] Ghosh, Z., Mallick, B. and Chakrabarti, J. (2009) Cellular versus viral microRNAs in host–virus interaction. Nucleic Acids Res. 37, 1035–1048.

[23] Roberts, A.P. and Jopling, C.L. (2010) Targeting viral infection by microRNA inhibition. Genome Biol. 11, 201.

[24] Reed, L.J. and Muench, H. (1938) A simple method of estimating fifty percent endpoints. Am. J. Hyg. 27, 493–497.

[25] Livak, K.J. and Schmittgen, T.D. (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(−Delta Delta C(T)) Method. Methods 25, 402–408.

[26] Fook, L.W. and Chow, V.T. (2010) Transcriptome profiling of host–microbe interactions by differential display RT-PCR. Methods Mol. Biol. (Clifton, NJ) 630, 33–47.

[27] Leong, W.F. and Chow, V.T. (2006) Transcriptomic and proteomic analyses of rhabdomyosarcoma cells reveal differential cellular gene expression in response to enterovirus 71 infection. Cell. Microbiol. 8, 565–580.

[28] Xu, L.J., Jiang, T., Zhang, F.J., Han, J.F., Liu, J., Zhao, H., Li, X.F., Liu, R.J., Deng, Y.Q., Wu, X.Y., Zhu, S.Y., Qin, E.D. and Qin, C.F. (2013) Global transcriptomic analysis of human neuroblastoma cells in response to enterovirus type 71 infection. PLoS ONE 8, e65948.

[29] Chen, C.S., Yao, Y.C., Lin, S.C., Lee, Y.P., Wang, Y.F., Wang, J.R., Liu, C.C., Lei, H.Y. and Yu, C.K. (2007) Retrograde axonal transport: a major transmission route of enterovirus 71 in mice. J. Virol. 81, 8996–9003.

[30] Chen, Y.C., Yu, C.K., Wang, Y.F., Liu, C.C., Su, I.J. and Lei, H.Y. (2004) A murine oral enterovirus 71 infection model with central nervous system involvement. J. Gen. Virol. 85, 69–77.

[31] Khong, W.X., Yan, B., Yeo, H., Tan, E.L., Lee, J.J., Ng, J.K., Chow, V.T. and Alonso, S. (2012) A non-mouse-adapted enterovirus 71 (EV71) strain exhibits neurotropism, causing neurological manifestations in a novel mouse model of EV71 infection. J. Virol. 86, 2121–2131.

[32] Lu, J., He, Y.Q., Yi, L.N., Zan, H., Kung, H.F. and He, M.L. (2011) Viral kinetics of enterovirus 71 in human abdomyosarcoma cells. World J. Gastroenterol. 17, 4135–4142.

[33] Lui, Y., Timms, P., Hafner, L., Tan, T., Tan, K. and Tan, E. (2013) Characterisation of enterovirus 71 replication kinetics in human colorectal cell line, HT29. SpringerPlus 2, 267.

[34] Park, H., Serti, E., Eke, O., Muchmore, B., Prokunina-Olsson, L., Capone, S., Folgori, A. and Rehermann, B. (2012) IL-29 is the dominant type III interferon produced by hepatocytes during acute hepatitis C virus infection. Hepatology (Baltimore, MD) 56, 2060–2070.

[35] Osterlund, P., Veckman, V., Siren, J., Klucher, K.M., Hiscott, J., Matikainen, S. and Julkunen, I. (2005) Gene expression and antiviral activity of alpha/beta interferons and interleukin-29 in virus-infected human myeloid dendritic cells. J. Virol. 79, 9608–9617.

[36] Pagliaccetti, N.E., Eduardo, R., Kleinstein, S.H., Mu, X.J., Bandi, P. and Robek, M.D. (2008) Interleukin-29 functions cooperatively with interferon to induce antiviral gene expression and inhibit hepatitis C virus replication. J. Biol. Chem. 283, 30079–30089.

[37] Sainz Jr., B. and Halford, W.P. (2002) Alpha/Beta interferon and gamma interferon synergize to inhibit the replication of herpes simplex virus type 1. J. Virol. 76, 11541–11550.

[38] Pierce, A.T., DeSalvo, J., Foster, T.P., Kosinski, A., Weller, S.K. and Halford, W.P. (2005) Beta interferon and gamma interferon synergize to block viral DNA and virion synthesis in herpes simplex virus-infected cells. J. Gen. Virol. 86, 2421–2432.

[39] Fuchizaki, U., Kaneko, S., Nakamoto, Y., Sugiyama, Y., Imagawa, K., Kikuchi, M. and Kobayashi, K. (2003) Synergistic antiviral effect of a combination of mouse interferon-alpha and interferon-gamma on mouse hepatitis virus. J. Med. Virol. 69, 188–194.

[40] D'Onofrio, C., Franzese, O., Puglianiello, A., Peci, E., Lanzilli, G. and Bonmassar, E. (1992) Antiviral activity of individual versus combined treatments with interferon alpha, beta and gamma on early infection with HTLV-I in vitro. Int. J. Immunopharmacol. 14, 1069–1079.

[41] Sainz Jr., B., LaMarca, H.L., Garry, R.F. and Morris, C.A. (2005) Synergistic inhibition of human cytomegalovirus replication by interferon-alpha/beta and interferon-gamma. Virol. J. 2, 14.

[42] Okuse, C., Rinaudo, J.A., Farrar, K., Wells, F. and Korba, B.E. (2005) Enhancement of antiviral activity against hepatitis C virus in vitro by interferon combination therapy. Antiviral Res. 65, 23–34.

[43] Larkin, J., Jin, L., Farmen, M., Venable, D., Huang, Y., Tan, S.L. and Glass, J.I. (2003) Synergistic antiviral activity of human interferon combinations in the hepatitis C virus replicon system. J. Interferon Cytokine Res. 23, 247–257.

[44] Lei, X., Liu, X., Ma, Y., Sun, Z., Yang, Y., Jin, Q., He, B. and Wang, J. (2010) The 3C protein of enterovirus 71 inhibits retinoid acid-inducible gene I-mediated interferon regulatory factor 3 activation and type I interferon responses. J. Virol. 84, 8051–8061.

[45] Lei, X., Sun, Z., Liu, X., Jin, Q., He, B. and Wang, J. (2011) Cleavage of the adaptor protein TRIF by enterovirus 71 3C inhibits antiviral responses mediated by Toll-like receptor 3. J. Virol. 85, 8811–8818.

[46] Lei, X., Xiao, X., Xue, Q., Jin, Q., He, B. and Wang, J. (2013) Cleavage of interferon regulatory factor 7 by enterovirus 71 3C suppresses cellular responses. J. Virol. 87, 1690–1698.

[47] Chi, C., Sun, Q., Wang, S., Zhang, Z., Li, X., Cardona, C.J., Jin, Y. and Xing, Z. (2013) Robust antiviral responses to enterovirus 71 infection in human intestinal epithelial cells. Virus Res..

[48] Liu, M.L., Lee, Y.P., Wang, Y.F., Lei, H.Y., Liu, C.C., Wang, S.M., Su, I.J., Wang, J.R., Yeh, T.M., Chen, S.H. and Yu, C.K. (2005) Type I interferons protect mice against enterovirus 71 infection. J. Gen. Virol. 86, 3263–3269.