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A Review on Gene Expression of Monkey Pox Virus Infected Cells and Control The Disease

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ABSTRACT

Human monkey pox is an emerging viral zoonotic disease. This is caused by monkey pox virus. Monkey pox has a clinical presentation like flulike symptoms characteristic rash fever, malaise, back pain, and headache. Primarily monkey transmission to human is believed to occur through direct contact with infected animals (or) possibly by ingestion of inadequately cooked flesh. The review article mainly discuss about orthopox virus genes have been shown to suppress anti-viral cell defenses, exploit host cell machinery and delay infection induced cell death. And control there are no licensed therapies to treat human monkey pox viral infection, however the small pox vaccine can protect against the disease. The management of monkey pox is mainly used this medications like Small Pox Vaccine, Cidofovir, Brincidofovir, Tecovirimat, Vaccinia Immune Globulin (VIG). Discontinuation of vaccination has given rise to increasing susceptibility to monkey pox viral infection. This lead to a fear of bioterrorism. Effective prevention limiting contact with infected patients (or) animal. Limiting the respiratory exposure to infected patients. Mortality and Morbidity rate of this virus is very rare conditions. This virus mainly prone to neonatal & pediatric patients, the hospital stay is minimum of 4 weeks.

Keywords: Benign epidermal Monkey Pox (BEMP), Democratic Republic of the Congo (DRC), Monkey Pox Virus (MPV), Polymerase Chain Reaction (PCR), Immunoglobulin M (IgM), Center for Disease Control and Prevention (CDC), Fold Change Ratios (FCR), Ingenuity Pathway Analysis (IPA), Vaccinia Immune Globulin (VIG).

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INTRODUCTION

Monkey pox is a viral disease that resembles smallpox, but unlike smallpox, is acquired from animals.[1] Monkey pox virus is endemic in western and central Africa, where it circulates in unknown animal hosts and emerges periodically to affect humans. The consequences range from asymptomatic infections to severe, fatal illness. This virus also causes illness in nonhuman primates, and outbreaks have been seen occasionally in primate facilities, in various parts of the world.[16] The only outbreak of human monkey pox reported outside Africa occurred in the United States in 2003. The virus entered North America in exotic African rodents imported as pets, and spread to pet prairie dogs, which were highly susceptible to infection.[8]This virus subsequently infected approximately 70 people who had been in contact with these animals.(8) Prompt diagnosis of monkey pox is essential, to prevent this disease from becoming established outside Africa.[11] In addition, it must be distinguished from smallpox, which has been eradicated from human populations but is a potential bioterrorist weapon[11].

Epidemiology:

Occurrence People living in or near the forested areas may have indirect or low-level exposure, possibly leading to subclinical infection. The disease is rare and only known to be indigenous to the rain forests of western and central Africa. It was first recognized in humans in 1970 after the eradication of smallpox, possibly because of the subsequent unmasking of the infection. Surveillance reports from 1981-1986 documented 338 cases in the DRC (out of a 1982 estimated population of 5 million). In the 1996-1997 outbreaks in the DRC, the attack rate was 22 cases per 1000 population. Human infection with monkey pox has not been reported in West Africa since 1978. However, monkey pox continues to exhibit a robust emergence in the DRC, with sporadic occurrences of disease in neighboring countries.[11] In 2003, 11 cases and 1 death were reported from the DRC and 10 cases with no deaths were reported from Sudan in 2005. In United States, no cases occurred until the late spring 2003 outbreak in the Midwestern states. Between May 16 and June 20, 2003, 71 suspected cases of monkey pox were investigated. Morbidity during a monkey pox infection. Monkey pox case-fatality rates in Democratic Republic of the Congo were ~10% among non-vaccinated individuals as compared to those people who were vaccinated against smallpox, and the vaccinated group was noted to have fewer lesions and generally less severe disease [8]. The disease was generally self-limited, with resolution in 2-4 weeks, depending on the severity of the illness. However, a small subset of patients, most commonly pediatric patients, had a more severe course, with several patients requiring ICU care [15].

Complications reported from African outbreaks include pitted scars, deforming scars, secondary bacterial infection, bronchopneumonia, respiratory distress, keratitis, corneal ulceration, blindness, septicemia, and encephalitis. African cases have mortality rates of 1-10%, with the highest rates occurring in children and individuals without vaccination. In general, the prognosis is related to the amount of exposure to the virus, host immune response, comorbidities, vaccination status, and severity of complications. Poxvirus infections have no racial predilection and the incidence is equal in males and females.

Etiology:

Monkey pox results from infection by the monkey pox virus, a member of the genus Orthopoxvirus in the family Poxviridae (subfamily Chordopoxvirinae). Two clades of monkey pox viruses, the West African and Congo Basin viruses have been identified[.9] The Congo Basin viruses are more virulent. Monkey pox virus is closely related to some other orthopoxviruses such as variola (smallpox) virus, and it cannot be distinguished from these viruses in some laboratory tests.[11] Monkey pox should not be confused with benign epidermal monkey pox (BEMP), a pox viral disease of primates caused by tan pox virus, an antigenically unrelated virus in the genus Yatapoxvirus of the family Poxviridae.

Clinical manifestations:

In humans, the symptoms of monkey pox are similar to but milder than the symptoms of smallpox. Monkey pox begins with fever, headache, muscle aches, and exhaustion. The main difference between symptoms of smallpox and monkey pox is that monkey pox causes lymph nodes to swell (lymphadenopathy) while smallpox does not. The incubation period (time from infection to symptoms) for monkey pox is usually 7−14 days but can range from 5−21 days.[16]

The illness begins with:

- Fever, Headache ,Muscle aches, Backache ,Swollen lymph nodes,Chills,Exhaustion

Within 1 to 3 days (sometimes longer) after the appearance of fever, the patient develops a rash, often beginning on the face then spreading to other parts of the body. Lesions progress through the following stages before falling off:[1]

Macules, Papules, Vesicles, Pustules

Scabs

Transmission:

The transmission of monkey pox virus between prairie dogs is still incompletely understood. In these animals, the virus or its nucleic acids have been found in skin lesions, urine, feces, and oral, nasal and conjunctival exudates. In terminal cases, it appears to be widely distributed in the tissues. Experimental infections have been established in prairie dogs by intranasal inoculation or contact with fomites (bedding from an animal with lesions). Aerosol transmission might also be possible; however, this is still not entirely certain, as the experimental design did not rule out the possibility of nose-to-nose contact between cages. Experimentally infected prairie dogs can shed monkey pox viruses until 21 days after inoculation. There is little published information on transmission routes in other small animal pets. Monkey pox virus has been found in most tissues of dormice, and limited evidence suggests that some small animals, such as dormice and Gambian giant pouched rats, might carry this virus for a few weeks or months.[14] Viral DNA was detected in the tissues, urine and feces of one dormouse for at least 6 months, but no viral antigens were found when this animal was euthanized. Whether such animals shed infectious virus is not known. Monkey pox virus can be transmitted to people in bites from animals, in aerosols during close contact, or by direct contact with lesions, blood or body fluids. In Africa, human outbreaks have often been linked to handling, preparing and eating wild animals. In the U.S., most cases occurred among people who had close direct contact with prairie dogs;[7] some infections were apparently acquired in scratches and bites, or through open wounds. Person-to person transmission can also occur. In people, monkey pox virus has been isolated for up to 18 days after the onset of the rash. Potential routes of transmission between people include contact with skin lesions or infectious body fluids, or aerosol transmission during prolonged face-to-face contact. Transmission between humans appears to relatively inefficient, and sustained person-to-person spread has not been reported. Until 2005, the longest documented chain was four serial

transmissions.[13] More efficient person-to-person spread, with six serial transmissions, was later reported from an outbreak in the Republic of Congo. It is possible that the efficiency of transmission differs between viruses.[15]

Incubation period:

Reported incubation periods are 4 to 13 days in experimentally infected black-tailed prairie dogs, 11 to 18 days in 3 prairie dogs infected by exposure to fomites, and 4 to 5 days in experimentally infected ground squirrels. [10]In two studies, experimentally infected cynomolgus monkeys developed clinical signs 3 to 7 days after aerosol exposure.

CLINICAL AND LABORATORY DIAGNOSIS:

The geographic location of the patient is important in the diagnosis of monkey pox, as the disease usually occurs in remote villages in the tropical African rain forests. Differentiation from chickenpox is important; the latter appears in successive crops so that lesions at various stages of development are visible at any time. In contrast with smallpox, the distribution of chickenpox is 'centripetal' with more lesions on the trunk than on the face and extremities. For definitive diagnosis, scabs can be forwarded to a reference laboratory where electron microscopy may confirm the presence of an Orthopoxvirus and differentiate this virus from varicella virus. The virus can be cultured in tissue culture and identified by DNA restriction analysis.[6] A viral culture should be obtained from an or pharyngeal or nasopharyngeal swab. A skin biopsy specimen of the vesiculopustular rash or a sample of the roof of an intact vesiculopustule should be analyzed.[14]Tissue for PCR of DNA sequence-specific for the monkey pox virus may be obtained. Paired sera for acute and convalescent titers may be analyzed. Serum collected more than 5 days for IgM detection or serum collected more than 8 days after rash onset for IgG detection was most efficient for the detection of the monkey pox virus infection. A Tzanck smear can help differentiate monkey pox from other non-viral disorders in the differential diagnosis. However, a Tzanck smear does not differentiate a monkey pox infection from smallpox or herpetic infections. Monkey pox cases were confirmed based on virus isolation or detection of the virus by polymerase chain reaction (PCR) from a clinical specimen (skin biopsy or throat culture). Individuals who presented with fever and rash within 21 days of exposure to monkey pox and had serum positive for orthopox immunoglobulin M (IgM). [14]The most reliable clinical sign differentiating monkey pox from smallpox and chickenpox is enlarged lymph nodes, especially the submental, submandibular, cervical, and inguinal nodes. Regarding exanthema, nonspecific lesions and inflammation of the pharyngeal, conjunctival, and genital mucosae have been observed.

Treatment:

The Centers for Disease Control and Prevention (CDC) recommended Smallpox Vaccination within 2 weeks, ideally before 4 days, after a significant, unprotected exposure to a diseased animal or a confirmed human case Data from the African outbreaks suggests that prior smallpox vaccination confers 85% protection from monkey pox viral infection. **Smallpox Vaccine, Cidofovir, Brincidofovir, Tecovirimat (ST-246), and Vaccinia Immune Globulin (VIG)** can be used to control a monkey pox outbreak.[7]Efficacy of vaccination was noted to be prolonged with protection noted even several years after vaccination, and the incidence of complications being reduced since human infection with monkey pox virus is a rare disease, no benefit would be derived from vaccination with Vaccinia virus. Furthermore, smallpox vaccination cannot be undertaken in populations with high prevalence of HIV infection because of the risk of serious complications[.8] Antiviral chemotherapeutic treatment is not a viable option in those remote places where the disease is likely to appear.

Prevention and control:

Improved infection control measures, including the regular screening, and isolation of newly infected animals will certainly help in preventing outbreaks among animals. Better hygiene habits are warranted to avoid spreading of the virus on fomites which then become a source for newer infections.[12]Vaccination with vaccinia virus could be choice to protect animals. Because infections have been reported in Asian monkeys mixed with primates from Africa, care must be taken to house these species separately. Anyone who has been exposed to monkey pox virus should avoid contact with animals, particularly rodents and non-human primates, to stop transmitting the virus [13]. During an outbreak, monkey pox viral spread may be controlled by quarantining (at least for 6 weeks from the date of the last exposure) the infected animals and tracing of their contacts. Areas where these animals have been kept should be cleaned and disinfected thoroughly. Adherence to specific instructions from the state or local health department or the CDC Web site is required.[12].

GENE EXPRESSION OF MPV INFECTED CELL:

MPV compromises host's biological activities with a dominating global down regulation:

Down regulation was reported as the hallmark of gene expression modulation in Vaccinia-infected human and in lymphocytes of Variola-infected cynomolgus macaques (Macaca fascicularis). More than 89% of the regulated genes or 2,407 genes exhibited steady down regulation in both 3 and 7 hpi time points, while only 295 genes or 10.92% of total regulated gene showed upregulation under the same statistical constraints.[11] Up- and down-regulated

genes exhibit varied temporal regulation distribution Because MPV exhibit well-defined temporal gene expression stages that were classified into early, intermediate, and late stages, we anticipated the host response to exhibit some sort of an analogous pattern.[15] This may be represented in variable temporal expression of regulated host genes, where a subset will exhibit a higher copy number at early time point followed by a decline at late time point as the inducing viral protein or gene decreases or disappears. We followed peaks of gene expression regulation for each of the differentially expressed genes across both time points by dividing gene expression fold change (FC) at late time point (7 hpi) by FC of the same gene in early time point (3 hpi). Calculated fold change ratios (FCRs) 7/3 hpi for all 2,407 down-regulated genes are plotted. Bars represent the average FCR for each consecutive 100 genes after sorting them according to their FCR values from smallest to highest for data clarity. A plot for all 295 upregulated genes was made following identical steps, but bars represent the average FCR of 10 sequential genes. Genes with FCR > 1 indicate a relatively greater regulation in later stages, while < 1 ratio points to greater regulation in early stage. Only 7.9% of the down regulated genes showed higher fold change early in the course of infection, while 51.1% of the up regulated genes exhibited similar temporal expression trend[.9]In contrast with down regulated genes, peaks of host gene up regulation were almost equally distributed across infection time points.

Functional gene clusters:

We used Ingenuity pathway analysis (IPA) to identify the main host functions and canonical pathways influenced by viral infection. The differentially expressed genes at 3, 7 hpi time points fell into diverse functional categories including enzymes, transcription regulators, kinases, phosphatases, peptidases, transmembrane receptors and G protein coupled receptors, transporters, translation regulators, and micro RNAs data filter and included only genes that exhibited ≥ 1.8 FC in addition to the t-test filter with P values < 0.05 already in place. A total of 1,013 and 1,720 genes from 3 and 7 hpi time points, respectively, met analysis thresholds and were used in a comparative functional analysis to identify unique and common influenced host functions for both time points[.9]Functions relating to cell signaling, cell cycle, cell death, transcriptional modification, and DNA processing were more significant in 3 hpi time point, and while protein synthesis and molecular transport functions were identified with significant only at 7 hpi, and RNA damage and repair was found to be unique to 3 hpi time point. Time-point independent functions exhibited comparable -log (P-value) in both time points and included metabolism of essential building blocks such as amino acids, lipids, and carbohydrates , and

other functions related to cell morphology, cellular development, small molecule biochemistry, and posttranslational modification.[8]

Canonical pathways analysis:

To identify the effect of viral infection on major biological processes in the cell, we examined potential enrichment of all differentially expressed genes in known canonical pathways. The two data sets were analyzed using IPA library of canonical pathways. Genes exhibited ≥ 1.8 FC and associated with a canonical pathway in the Ingenuity pathways knowledge database were considered for the analysis. Both data sets contained.[15]

Molecular Mechanisms of Cancer (MMC):

370 molecules localized to cell membrane, mitochondrion inner and outer membrane, cell cytoplasm, and cell nucleus, this pathway is considered one of the most complex known pathways. Forty-eight genes of data set 7 hpi clustered to MMC to cover 13% of total pathway molecules with P-value of 1.25e-5, making it the first pathway influenced by MPV infection. The 3 hpi data set showed lower gene ratio of 9.5% but with a tighter correlation at lower P-value of 2.82e-6 . MMC pathway can be triggered by diverse stimuli including hormones, growth factors, cytokines, and oncogenes. Each of these stimuli is capable of inducing a variety of biological processes relating to cancer, including cell cycle regulation and cell cycle checkpoints, which were recognized in our analysis as independent pathways, and therefore will be discussed separately.[10] The majority of the genes specific to cancer mechanisms pathway are involved in apoptosis regulation. Identified modulation in expression patterns of these genes suggests signaling apoptosis in infected cells, e.g., Noxa or PMAIP1 (phorbol-12-myristate-13-acetateinduced protein 1) shows more than 44-fold up regulation at 7 h PI. This molecule inactivates the anti-apoptotic molecule Bcl2 (B-cell CLL/lymphoma 2), which regulates mitochondrial membrane potential by controlling the voltage dependent anion channel (VDAC) , consequently increasing the mitochondrial membrane permeability and the release of cytochrome C that serves as a major apoptosis inducer . In addition to Bcl2 inactivation, the expression of Bcl2 gene itself exhibited a significant -2.83-fold suppression, which would enhance apoptosis signaling. Apoptosis-induced cysteine-aspartic acid peptidases (caspases) such as caspase 3, which enhance the degradation of Bcl2 and trigger subprograms for cell dismantling and removal, exhibited 3.2-fold up regulation. Caspase 7, 8, and 10 expression remained unchanged, and only caspase , which functions as a downstream enzyme in the caspase activation cascade, 3, 7 hpi time points, respectively. Other pro-apoptotic genes like BBC3 (BcL2 binding component 3) or PUMA and p21 protein (Cdc42/Rac)-activated kinase 2 or PAK2 showed similar

suppression trend of -1.85 and -1.99 FC at 7 hpi, respectively. Proteosomal degradation has been associated with apoptosis. [11]Our results show a 4.05 FC up regulation of nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, epsilon NFKBIE, which mediates cytoplasmic sequestering of transcription factor and acts as a transcription repressor. The effect of NFKBIE up regulation on apoptosis is unclear because it was reported to exhibit a cell line dependence . Cell division cycle 25 homolog A, B, and C expression showed a remarkable regulation; e.g., Cdc25, which plays a key role in promoting progress through S phase , showed 1.7 FC, while Cdc 25 B and C, which regulate and promote entry into mitosis , exhibited -4.3 and -1.92 FC, respectively. Similarly, cyclin -dependent kinase regulators, cyclin E2 and cyclin D1, which are strongly tied to a variety of cancers , showed up regulation of 3.2 and 2.2 FC, respectively. Other cell cycle regulators that have overlapping functions with apoptosis mechanisms showed sharp down regulation. This include members of the p21 RAS group such as KRAS (v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog) and Ras homolog or the RHO GTPase protein group including RHOB, RHOQ, RHOT1, and RND[8].

This includes intersect in 1 (SH3 domain protein) gene, which encodes a cytoplasmic membrane-associated protein that indirectly coordinates endocytic membrane traffic with the actin assembly machinery, Rho-effector ROCK1 serve a number of key cellular functions, such as morphological differentiation and cell motility which are closely associated with changes in cytoskeletal dynamics . Additionally, RAS p21 protein activator (GTPase activating protein) , v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog and SOS2 are crucial genes in polymerization of actin filaments and cytoskeleton reorganization.[11] Ion channels represent an intriguing and novel class of genes that were impacted by MPV infection. We identified 10 genes encoding nine ion channels and a transporter that underwent increasing suppression during infection. Most of these channels localize to cell membrane, and collectively contribute to transport of all essential ions involved in maintenance of cell membrane potential and osmolality homeostasis. While mechanisms of transport modulation have been described previously, as in the indirect consequences of Ras, Rho, and Rab small GTPases regulation, its effect on viral infections and global cell biology remain unclear except for a recent report describing the interaction of myxoma poxvirus protein M11L with mitochondrial permeability transition pore and its role in delaying apoptosis in host cells.[9] The down regulation trend of channel expression identified here pose many intriguing questions, especially in the light of evolving evidence in support of ion channels role in virus release and infected cells rupture . Progression of the cell cycle is tightly regulated process with many redundant checkpoints that ensure proper

transition across cell cycle phases.[8]Our results showed significant modulation in the expression of many genes that play essential roles in cell cycle regulation, which led to the identification of ATM signaling, G2/M DNA damage checkpoint, regulation by BTG family protein, and G1/S checkpoint as major influenced pathways during MPV infection. A core cell cycle regulation gene, Cdc25 kinase, has three essential homologs Cdc 25A/B/C that exhibited significant regulation upon MPV infection. While Cdc25B/C showed down regulation favoring cell arrest in G2 phase, Cdc2A exhibited up regulation, favoring S phase progression. [10]The impact of this mode of cell cycle regulation on viral infection remains unknown. However, cell arrest in G2 phase was described in other viral infections including human immunodeficiency virus (HIV) and was found to be mainly mediated by viral protein R (Vpr) . While many of the G2/M DNA damage checkpoint pathway genes are known to be modulated during HIV infection, Vpr seems to induce cell arrest by molecular mechanisms other than the classic DNA check point Recently, evidence supporting a role for PP2A in Vprinduced arrest has emerged, and was substantiated further by other studies in support of PP2A being a common target during infection with other viruses, including simian virus 40 (SV40), polyoma virus, human T lymph trophic retrovirus and adenovirus . Our results showed significant down regulation in two PP2A isoforms, regulatory subunit B' gamma isoform (PPP2R5C) and protein phosphatase 2, regulatory subunit B' epsilon isoform (PPP2R5E), suggesting that the induction mechanisms of G2 arrest in MPV infection might be similar to those observed in other viruses. Because genetically diverse viruses seem to induce the same G2 arrest response in different infected cells, it is likely that this response has an important function and might be part of antiviral host defenses. While some of the viral genes eliciting this response are being identified as Vpr in HIV, and E4 of F4 and HTLV tax protein in adenoviruses, the MPV gene inducing this response remains unknown. Other important genes in cell cycle regulation showing expression favoring progression of cell cycle and arrest only in G2 phase include Rb, E2F, cyclins, BTG1, and BRCA1.[9]In this study we combined microarray with data mining and statistical analysis to identify important interfaces of host-pathogen interaction. Our results aligned nicely with previous reports carried out using viruses from the same or different genus, and provided new set of genes that play important roles in MPV infection. Further work is warranted to validate and examine the potential of these genes in antiviral therapies. [10]

Conclusion: Smallpox has been eradicated from the human population since 1980, there is the potential for monkey pox to fill this void. An extended chain of person-to-person transmissions of monkey pox in 2003 in the Republic of Congo reveals the potential of further adaptation of

the virus to become a more successful human pathogen. Based on the genome comparisons between the Central and West African strains of monkeypox virus, it is interesting to speculate that the ortholog to COP-C3L, a gene coding for an innate immune response protein (a complement control protein), may contribute to the difference in virulence between the more virulent and less virulent strains of monkeypox virus(17). The Central African strain of monkeypox also contains truncated orthologs of COP-E3L and COP-K3L, two proteins that function in IFN resistance, that are full-length in variola and vaccinia viruses. In contrast to COP-E3L and COP-K3L, the ortholog to BR-203 (a protein that prevents apoptosis of infected lymphocytes) is full length in monkeypox virus. Monkeypox infection is an important emerging pathogen that, based on serologic studies in Africa, may result in more infections than originally believed. If a virulent strain of monkeypox were introduced in a setting where individuals have no immunity to orthopoxviruses, this may provide the virus with the opportunity to exploit this naive population, which could lead to an epidemic.

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