Measles virus: Background and oncolytic virotherapy

Sankhajit Bhattacharjee, Pramod Kumar Yadava*
Applied Molecular Biology Laboratory, School of life Sciences, Jawaharlal Nehru University, New Delhi 110067, India

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ABSTRACT

Measles is a highly transmissible disease caused by measles virus and remains a major cause of child mortality in developing countries. Measles virus nucleoprotein (N) encapsidates the RNA genome of the virus for providing protection from host cell endonucleases and for specific recognition of viral RNA as template for transcription and replication. This protein is over-expressed at the time of viral replication. The C-terminal of N protein is intrinsically disordered, which enables this protein to interact with several host cell proteins. It was previously proved in our laboratory that N expressing human cancerous cells undergo programmed cell death because of reactive oxygen species (ROS) generation as well as Caspase 3 activation. The phosphoprotein (P) along with N protein enclosed viral genomic RNA forming a ribonucleoprotein complex (RNP). It also establishes interaction with the large protein (L) i.e. viral RNA dependent RNA polymerase to ensure viral replication within host cells. The host cell receptors of this virus are CD46, SLAM/CD150 and PVRL4. Measles virus is latently oncotropic in nature and possesses oncolytic property by syncytia formation. We try to highlight the application of this property in developing a virotherapeutic vehicle.

1. Introduction

Measles virus belongs to the genus Morbillivirus under family Paramyxoviridae of the order Mononegavirales. The Paramyxoviridae family also includes mumps virus and other viruses causing infections in respiratory tract. Measles is transmitted either through air or by direct contact with body secretions. The primary site of viral infection is respiratory tract. Then it disperses throughout the body viz. lymphoid tissue, liver, lungs, conjunctiva, and skin. This virus infects humans only [1,2]. Death is common among young children (below 5 years) and occurs due to measles related complications viz. infections in respiratory tracts like pneumonia, brain swelling or encephalitis, blindness and ear infections, dehydration and diarrhoea. Recovery from measles makes an individual immune for rest of one’s life [3]. The worldwide death rate due to measles is decreased amazingly due to routine vaccination and mass campaigning. The measles vaccine is often injected along with rubella and/or mumps (MMRV: Measles Mumps Rubella Vaccine). Compared to 73% in 2000, 85% of world’s children had received a vaccine dose on their first birthday 2013 [4].

2. Viral structure

Measles virus is generally of round shape but shows pleomorphism i.e. the appearance of two or more distinctly different forms in the life cycle of some organisms. It contains single-stranded RNA of negative polarity. It is an enveloped virus with non-segmented genome. The RNA genome is 15,894 nucleotides in length and forms complex with N protein. The arrangement is helical and follows the “rule of six” [5]. The genome contains a 52 nucleotide non-coding leader region at the beginning that acts as a transcriptional promoter and a 37 nucleotide non-coding trailer region at the end that acts as a transcriptional terminator. The MV genome organization bears essential similarity to other members of Paramyxoviridae. The genome contains six genes code for eight viral proteins. The anti-genome structure is S′-N, P, M, F, H, L-3′. V and C are two accessory proteins produced by editing and use of an alternative ORF respectively from P gene sequence [6].

The N protein is coded by N gene. The N terminal region of this protein is conserved and is called N CORE. It is 400 amino acids long. The C-terminal- known as NTAIL is intrinsically disordered, which enables it to establish interaction with many viral and host cell proteins. The C-terminal domain of P protein and matrix protein (M) are its interacting partners [7]. The host cell partners that were reported to interact with N tail are Heat shock protein (Hsp72), eukaryotic translation initiation factor 3 (eIF3-p40) and Interferon Regulatory Factor (IRF3) [8]. Nucleocapsid protein provides protection to the viral RNA. The N enclosed RNA is a template for viral multiplication.

The P, V and C proteins are coded by P gene. V is the result of RNA editing and C is coded from an alternative reading frame [9]. These proteins hardly play any role in maintaining viral structure but act as virulence factors to suppress innate immune response in host by
interfering with Interferon (IFN) signaling pathway [10]. The P protein establishes connection with both N and L proteins to ensure proper replication and transcription. It acts as a cofactor and is a part of ribonucleoprotein (RNP) complex [11].

The hydrophobic M protein is coded by M gene. Though it is not a membrane protein, its association with the membrane has been reported [12]. It fills the spaced between lipid envelope and nucleocapsid core. This protein interacts with RNP complex as well as cytoplasmic tails of H and F proteins. Interestingly, this is necessary for cell fusion. It has an inhibitory effect on viral polymerase and takes part in viral lifecycle and organizing viral structure [13].

Two glycoproteins that protrude out of viral envelope, are coded by fusion (F) and hemagglutinin (H) genes respectively. The H protein recognizes, hence attaches with the host cell receptors like SLAM/CD150 and CD46, thereby determining viral tropism. Only after successful attachment, fusion to the host cell membrane is mediated by F protein. This protein undergoes structural transformation only after a successful attachment [14,15].

The viral polymerase (an RNA-dependent RNA polymerase, RdRp) or large protein is coded by the L gene. It catalyzes RNA synthesis, polymerization, capping, polymerization, methylation and polyadenylation. The negative sense RNA is transcribed directly into mRNA without forming any DNA intermediate [16] (Fig. 1).

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3. Receptors

Measles virus interacts with three host cell receptors viz. CD46, SLAM/CD150 and PVRL4.

The cluster differentiation marker CD46 is a complement regulatory protein. The CD46 gene is located on chromosome 1q32. It acts as a complement receptor as well as an inhibitor [17]. This type I membrane protein plays a key role in regulating the complement system. It acts as a cofactor of serum factor I, which mediates the cleavage of complement components viz. C3b and C4b, thereby protecting the host cells [18]. The action of this protein is necessary at the time of human fertilization process. It is a receptor molecule for Edmonston vaccine strain of MV, human herpesvirus-6 (HHV-6) and type IV pili of Neisseria [19]. The extracellular domain is composed of four short consensus repeats (SCR1-4), containing 60 amino acids. This domain is a closely packed beta-barrel bordered by flexible loops. The receptor contains a STP (Ser/Thr/Pro) domain, a helical transmembrane domain and an intracellular cytoplasmic domain, which takes part in signal transduction [20]. CD46 is expressed at basal level in all nucleated cells but is over expressed in all adenocarcinoma cells. This receptor has a predominant role in protecting the tumor cells from host immune response. The vaccine of measles Edmonston strain can be used for treatment of adenocarcinoma as CD46 is proved to be the receptor of entry of this strain. The interacting partners of CD46 are CD9, CD29 and CD151 [21].

Signaling lymphocytic activation molecule (SLAM) or CD150 is encoded by SLAMF1 gene located on chromosome 1q22 in humans. The interacting partners of SLAM are SH2D1A, SH2D1B and PTPN11 [22]. This glycoprotein is expressed on the surface of certain immune cells viz. dendritic cells, natural killer cells, B and T cells. The wild type strain of measles virus recognizes CD150 as its receptor. It also functions as a co-activator on B and T cells. It possesses two extracellular domains, which correspond to variable (V) and constant (C) regions of immunoglobulin super family and an endodomain containing an SH2 binding region. The successive duplications in common ancestral gene resulted in the formation of molecules of CD150 family. The wild type vaccine strain cannot kill cancer cells unless it is attenuated [23].

Nectins, belonging to the family of cell adhesion molecules, play a role in adhesion in chemical synapse of neuronal tissues and adherens junction of epithelial tissue. The cellular adhesion mediated by nectins is Ca²⁺-independent [24]. Four nectin molecules have been identified so far, they are nectin-1, nectin-2, nectin-3 and nectin-4, in humans. Each molecule is composed of an intracellular domain, a single transmembrane domain and three extracellular immunoglobulin domains; the latter contain two constant regions and one variable region. The intracellular domain interacts with afadin, a scaffold protein, to form dimers. The interactions are homophilic or heterophilic and cadherin molecules are recruited for strengthening binding affinity. The H protein of measles virus interacts specifically with the V (variable) domain of Nectin 4/PVRL4 (primary vitreoretinal lymphoma). In many adenocarcinoma cell lines it is over expressed on the apical surface and can be a potential therapeutic target [23,25] (Fig. 2).

4. Viral replication

The strategy of measles replication and transcription bears similarity with other Paramyxoviridae, which is placed in group V according to Baltimore classification. The viral polymerase recognizes RNP complex as its template to initiate viral replication and transcription [26]. The process of transcription starts at 3′ end and continues towards the 5′ end of the genomic RNA template. The synthesized mRNA is translated into proteins. The virus utilizes host translation machinery to ensure proper multiplication. The
complementary positive strand is also synthesized from negative-sense genome by viral polymerase for production of more copies of the genome. The N protein accumulation is essential for switching from transcription to replication process [27].

Following the process of transcription and replication, the viral molecules assemble for generation of fully infectious particles. The C-terminal of N protein establishes interaction with M protein, this was confirmed by co-immunoprecipitation and yeast two-hybrid assay. The M protein plays an important role in incorporating the RNP into virions. It also assists in linking RNP with those parts of host cell membrane, where viral glycoproteins are introduced. An ESCRT-independent Virion release independent of endosomal sorting complex required for transport (ESCRT) has also been reported [28].

The phenomenon of polar attenuation occurs in measles virus- the gene expression at 3′ end of the genomic RNA is greater compared to those at 5′ end [29] (Fig. 3).

5. Immune suppression due to viral infection

Measles virus infection causes transient immune suppression that renders the affected individual more susceptible to other pathogenic infections. It causes lymphopenia, where the count of both B and T cells decreases extensively. The cytotoxic T-cells play a role in viral clearance. The appearance of CD4+ and CD8+ T lymphocytes is a unique feature of this response [34].

6. Oncolytic measles virus and virotherapy

Cancer, a group of diseases, is characterized by abnormal and uncontrolled cell growth as well as cell migration and invasion. Cancer cells are capable of escaping apoptosis. The tumors are viewed as either benign or malignant. The benign tumors are localized at the point of origin and never invade other parts of body but the malignant ones invade tissues away from the point of origin via blood or lymph- a phenomenon called metastasis [35]. Cancers can be classified based on the tissue of origin into the following types- 1) carcinoma (cancer of epithelial tissue), 2) sarcoma (cancer of connective tissue), 3) lymphoma and leukemia (cancer of hematopoietic cells), 4) germ cell tumor (cancer of pluripotent cells) and 5) blastoma (cancer of embryonic tissue or precursor cells [36].

In 1954 Enders and Peebles isolated measles virus from a patient, named Edmonston [37]. The vaccine against measles was developed after multiple passages. Further passages generated Schwarz and Moraten strains, which are used today [38]. These vaccines are used for protection of millions all over the world successfully. These live-attenuated vaccine strains possess oncolytic activity and are effectively used in clinical trials [39]. As CD46 is over expressed in many tumor cells, it becomes the preferred target of the virus. Recently it has been reported that nectin-4 also acts as a receptor for both wild type and vaccine strains. The unmodified attenuated viral vaccine strain (Edmonston Vaccine Strain) has been used to treat several cancers viz. leukemia, myeloma, ovarian and breast cancer. Genetically engineered measles viruses are utilized for oncolytic virotherapy after “blinding” it to its natural receptors. Table 1 presents the genetically modified viruses used against cancer [38,39]. MV-H protein is solely associated with viral tropism, so the latter is modified by inserting at the C-terminal of H protein the ligands of the receptors displayed by the cancer cells. The ligand is a single chain antibody for the receptors e.g. CD20, CD38, CD133, epidermal growth factor receptor (EGFR) and carcinoembryogenic antigen (CEA) [40–42]. When the cancer cells are infected with MV-CEA, the soluble peptide CEA is released into the blood stream. Thus CEA level can be detected in serum of patients, treated with MV-CEA vaccine [43]. Purine nucleoside phosphorylase (PNP), found in E. Coli, act as a produg convertase to assist conversion of chemotherapeutic prodrugs such as fludarabine into 2-fluoroadenine, a highly toxic metabolite, which intercalates in DNA to promote apoptosis. Recombinant measles virus, expressing produg convertase, was generated [44]. To ensure invasion and metastasis, the cancer cells secrete matrix metalloproteinases (MMP) to carry out degradation of extracellular matrix. Recombinant measles virus with MMP- cleavable sequences in place of furin-cleavable site of F protein induces the virus to fuse selectively with MMP-expressing cancer cells [45]. For quantifying tumor development and metastasis, the tumor cells are...
transduced with recombinant MV containing LacZ reporter gene to quantify beta-galactosidase enzymatic activity that is reported to be higher in invading cancer cells [46,47]. Recombinant measles virus expressing neurophil activating protein (NAP), a H. Pylori protein, stimulates the release of proinflammatory cytokines to boost anti-cancer immune response [48]. Iodine uptake in thyroid gland is mediated by a membrane ion channel called thyroidal sodium iodide symporter (NIS). Genetically modified measles expressing this ion channel are capable of inducing iodine symport, hence increased iodine concentration within cancer cells [49–52]. The expression of programmed death ligand 1 or CD274 is up regulated in cancer cells to equip the latter to evade anti-tumor immune response. When oncolytic virotherapy is combined with immune checkpoint modulators, the tumor burden has been reported to be reduced by direct cell lysis and anti-tumor immunity is stimulated. Genetically modified measles vaccine strain encoding PDL1 antibody is found to be an efficient oncolytic agent against melanoma [53]. Recombinant measles vaccine strain encoding human lambda light immunoglobulin chain (MV-lambda) is used effectively against multiple myeloma. The myeloma cell, which over-expresses human kappa light immunoglobulin chain (one kappa and one lambda chain) upon infection. This converted marker is quantified easily by immunological techniques [54]. Granulocyte macrophage colony stimulating factor (GM-CSF) by genetically engineered measles vaccine strain has been reported to cause an up regulation of anti-tumor response by neutrophil [55]. The outcomes of clinical trials are encouraging [39]. The cancer cells are destroyed by syncytia formation mediated by the viral fusion (F) protein.

9. Conclusion

Once the scientists were engrossed in designing an effective vaccine against measles but now measles Edmonston vaccine strain is being utilized as an effective therapeutic vaccine against cancer. Clinical trials are ongoing and the response is optimistic. Besides chemotherapy and radiotherapy, virotherapy has become an effective tool to treat malignancy. The genetically engineered virus that can boost the cancer suppressed immune system has been developed. The anti-tumor activity of CD8+ NKG2D+ cells is enhanced up on infection of hepatocellular carcinoma cells with Edmonston vaccine strain of measles virus and CD8+ NKG2D+ cells induce apoptosis via extrinsic pathway [60]. Other viruses of Paramyxoviridae family also possess this character and their oncolytic property has been studied vividly. However it should be kept in mind that long term vaccine against wild type measles has not yet been developed. The virus, which possesses a property to fight against cancer, is still one of the major causes of child mortality.

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Appendix A. Transparency document

Transparency document associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bbrep.2017.12.004.

References

[1] L. Biebrroewc, et al., Viral exanthems: an update, Dermatol. Ther. 26 (6) (2013) 433–438.
[2] M. Ludlow, et al., Pathological consequences of systemic measles virus infection, J. Pathol. 235 (2) (2015) 253–265.
[3] Y. Huijing, et al., Vitamin A for treating measles in children, Cochrane Database Syst. Rev. 4 (2005) (CD001479).
[4] E. Leuridan, et al., Measles outbreak in Europe: susceptibility of infants too young to be immunized, Vaccine 30 (41) (2012) 5905–5913.
[5] J.M. Bourhis, et al., The intrinsically disordered C-terminal domain of the measles virus nucleoprotein interacts with the C-terminal domain of the phosphoprotein via two distinct sites and remains predominantly unfolded, Protein Sci. 14 (8) (2005) 908–919.
[6] P. Devaux, et al., Measles virus P protein gene products: conformational flexibility of the P/V protein amino-terminal domain and C protein infectivity factor function, J. Virol. 78 (21) (2004) 11632–11640.
[7] S.A. Krumm, et al., The measles virus nucleocapsid protein tail domain is dispensable for viral polymerase recruitment and activity, J. Biol. Chem. 288 (41) (2013) 29943–29953.
[8] C. Pohl, et al., Measles virus M and F proteins associate with detergent-resistant membrane fractions and promote formation of virus-like particles, J. Gen. Virol. 88 (Pt 4) (2007) 1243–1250.
[9] M. Iwasaki, et al., The matrix protein of measles virus regulates viral RNA synthesis and assembly by interacting with the nucleoprotein, J. Virol. 83 (20) (2009) 10374–10383.
[10] M.D. Kahlbeck, et al., The measles virus fusion protein transmembrane region modulates availability of an active glycoprotein complex and fusion efficiency, J. Virol. 82 (22) (2008) 11437–11445.
[11] C.K. Navaratnajah, et al., The heads of the measles virus attachment protein move to transmit the fusion-triggering signal, Nat. Struct. Mol. Biol. 18 (2) (2011) 128–134.
[12] B.M. Blumberg, et al., Measles virus L protein evidences elements of ancestral RNA polymerase, Virology 164 (2) (1988) 487–497.
[13] M.K. Liszewski, et al., Membrane cofactor protein (MCP or CD46): newest member of the regulators of complement activation gene cluster, Annu. Rev. Immunol. 9
