REVIEW

EXPERIMENTAL ONCOLOGY

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D. DAHRIZAL [1, *, †], IBRAHIM M. AZIZ [2, †], GANI ASA DUDIN [1], R. BHAT [2]

[1] Politeknik Kesehatan Kemenkes Bengkulu, Indonesia
[2] College of Science, King Saud University, Riyadh, Saudi Arabia

* Correspondence: Email: ganiasa@gmail.com

† Have equal contribution

ONCOLYTIC ACTIVITY OF HUMAN RESPIRATORY SYNCYTIAL VIRUS

Oncolytic viruses (OVs) are emerging as novel tools in cancer therapy. Oncolytic virotherapy offers an attractive therapeutic combination of tumor-specific killing and immune co-stimulation, therefore amplifying the host immune response against tumors. Moreover, OVs can be engineered for the expression of different immunostimulatory molecules to optimize and enhance the efficacy of oncolytic virotherapy. The effectiveness of OVs has been demonstrated in many preclinical studies for different types of cancers to achieve the aim of personalized cancer therapy. Human respiratory syncytial virus (RSV), an RNA virus of the *Pneumoviridae* family causes severe lower respiratory tract infections in infants and immunocompromised individuals. Interestingly, the oncolytic activity of RSV demonstrated in human prostate, hepatocellular, and dermal cancer cells is mostly mediated via apoptotic cell death associated with the impaired NF-κB activation or with the defect of the IFNα/β-induced STAT-1 activation. At the same time, the studies on cervical cancer revealed that RSV infection resulted in autophagy activation and apoptosis through the ROS-BAX and TNF-α-mediated pathways. The rational combinations of OVs, including RSV, with other approaches may benefit patients whose response to conventional therapies is limited. Here, we discuss the oncolytic activity of RSV and its potential use against different types of cancer.

Keywords: RSV, oncolytic virotherapy, oncolytic viruses, immunotherapy, immune activation, tumors.

Cancer is a leading cause of death worldwide. The current cancer treatment usually involves radiotherapy, chemotherapy, and immunotherapy, but effective and precise therapy is still lacking. Besides, some tumors are difficult to operate and are resistant to radio- and chemotherapy, so the development of novel therapeutic strategies with the least adverse outcomes is required. In the recent past, cancer immunotherapy has made immense progress with the development of innovative drugs and therapies. Noteworthy, the oncolytic viruses (OVs) have emerged as a decisive tool for immunotherapy approaches against cancer. Nonetheless, concerns about viral pathogenicity and toxicity have limited the feasibility of oncolytic virotherapy. However, recent advances in bioengineering have improved upon a new generation of OVs with enhanced safety and efficacy [1].

OVs are replication-competent viruses that selectively replicate in tumor cells without damaging healthy cells. OVs not only eliminate tumor cells but also stimulate anti-tumor immune responses.

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The lysis of tumor cells results in the release of pathogen-associated molecular patterns (PAMPs), tumor-associated antigens (TAAs), etc., which in turn activate antigen-presenting cells (APCs) thereby stimulating adaptive immune responses [2]. The numerous OVs have been tested as wild or genetically modified for use in cancer virotherapy, including adenoviruses, herpes simplex virus, vaccinia virus, measles virus, Newcastle disease virus, vesicular stomatitis virus, myxoma virus, parvoviruses, etc. [3]. However, oncolytic virotherapy has limitations due to the host antiviral immune response, which targets virus-infected cells thereby limiting the effectiveness of oncolytic virotherapy. Various strategies have been employed to overcome this problem. Notably, host immune cells are loaded with OVs, which protects from immune neutralization and effectively targets the virus at the tumor site. Another strategy involves the inclusion of immunomodulatory molecules like cytokines to increase tumor selectivity and antitumor effects [4].

A large number of preclinical and clinical trials exploring oncolytic virotherapy are undergoing in combination with other conventional cancer therapies such as radiotherapy and chemotherapy. Specifically, the combination of OVs with CAR-T or NK-cell therapy may further improve the prospects of this mode of therapy by overcoming intratumoral barriers [2—4].

Mechanisms of action. OVs demonstrate a natural ability to target cancer cells selectively while sparing normal cells. OVs utilize the hallmarks of cancer cells such as immortality, defect in antiproliferation signals, evasion of apoptosis, increased angiogenesis, and metastasis, unlike normal healthy cells. Moreover, tumor cells overexpress certain surface receptors to which OVs selectively bind with the following cell infection. Additionally, OVs exploit aberrant signaling pathways that maintain sustained cancer growth to replicate within cancer cells. OVs can also replicate in tumor tissues in a hypoxic environment [5].

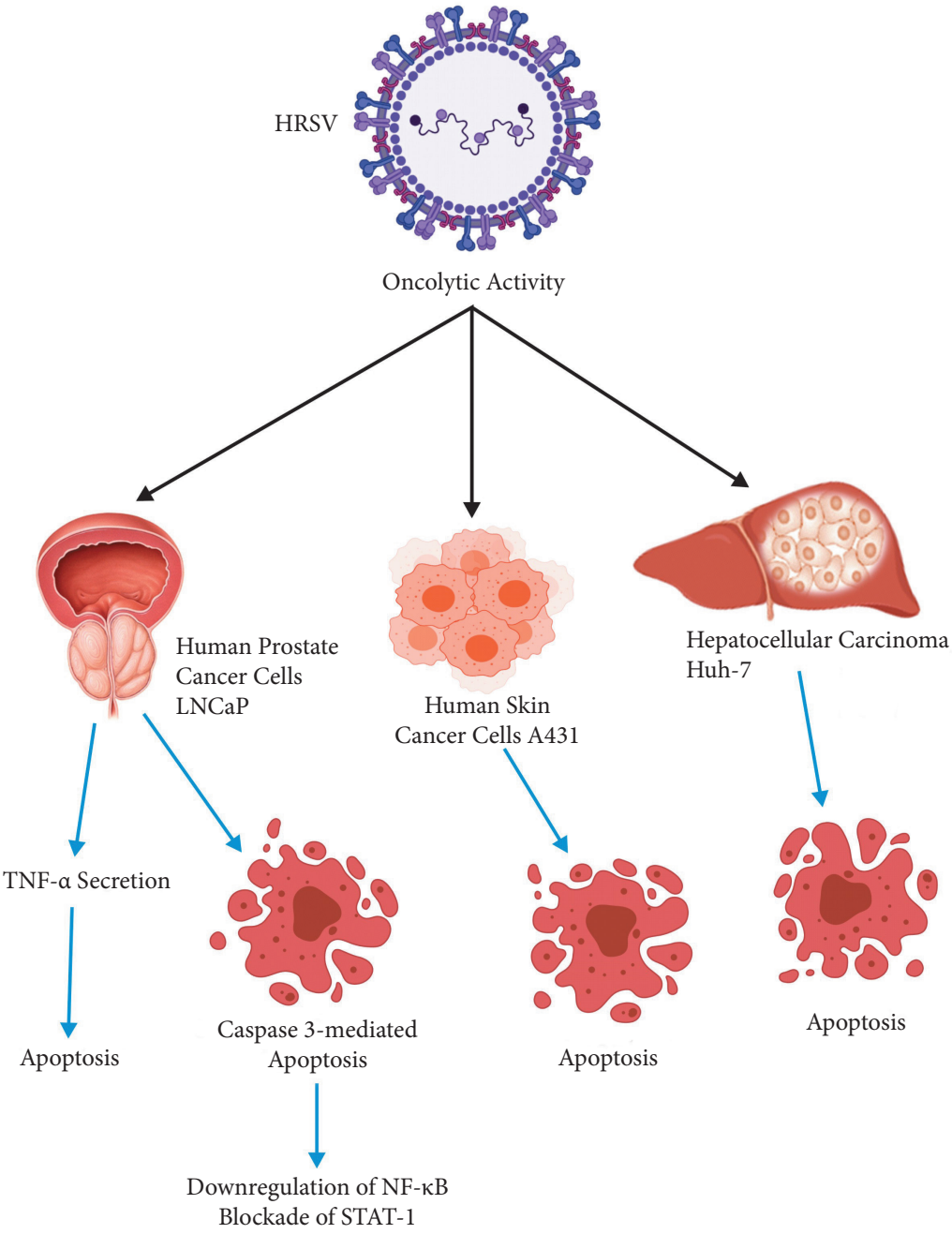
OV therapy relies on a dual strategy of selectively infecting tumor cells and then stimulating antitumor activity through the release of tumor antigens and immunostimulatory molecules. Upon infection by OVs, the lysis of tumor cells leads to the release of viral and cellular antigens and cytokines, which ensues a secondary anti-tumor immune response. The activation of the APCs recruits $CD4^+$ and $CD8^+$ cells to destroy the cells expressing viral antigens in tumors.

OVs can also be engineered to deliver immunostimulatory cytokines or suicide genes to enhance tumor cell lysis. Much of the emerging research on OVs now focuses on the strategies to amplify their immunogenicity and tumor destruction through the insertion of genes for IL-2, IL-12, GM-CSF, etc. This can improve tumor cell destruction by enhancing entry and direct killing of cancer cells along with the recruitment of the host's immune cells to the tumor site [2, 6].

The cell's ability to tolerate viral infection may vary depending on the selectivity of RSV permissiveness. In the case of non-tumorigenic cells, upon viral infection, the host functional immune system reacts by triggering the transcription factor STAT-1, which is activated by type-I interferon (IFNα/β). This transcription factor is necessary for the expression of the antiviral genes and, as a result, limiting the spread of infection. According to Echchgadda [7], RSV-induced oncolysis is effective in both LNCaP prostate cancer cells and RM1 murine prostate cancer cells. While LNCaP cells were capable of producing IFN in response to RSV infection, they cannot activate the type-I interferon (IFNα/β)-induced transcription factor STAT-1, which is necessary for the antiviral gene expression. The prostate cancer cells have a higher rate of RSV replication compared to cell lines originating from the normal prostate [7].

It is still unclear whether RSV plays any role in the signaling pathways that lead to apoptosis, and more research is needed to determine whether RSV exerts an oncolytic effect on various cancer types. Comparably, Salimi [8] has found that RSV was implicated in the activation of apoptosis and the suppression of skin cancer cell proliferation, which correlated with an increased viral load and oncolytic cell death. Notably, while the majority of studies involving other viruses exploit genetically modified or engineered viruses, RSV studies use the original strains of the virus.

Human RSV as an oncolytic virus. Human RSV (HRSV) is an enveloped, non-segmented negative-sense single-stranded RNA virus classified in the genus *Orthopneumovirus* of the newly created *Pneumoviridae* family within the order *Mononegavirales* [9]. HRSV is a major cause of severe lower respiratory tract infections in infants, children, el-



Oncolytic activity of RSV. RSV inhibits cell growth and induces apoptosis in a few hepatocellular carcinoma and human skin cancer cells. RSV possesses oncolytic activity against prostate cancer cells because of their defective NF-κB pathway and causes caspase-3-mediated apoptotic death. RSV induces TNF-α production in RSV-infected cells resulting in apoptosis of tumor cells. RSV induces apoptosis in infected prostate cancer cells by utilizing their deficiency in the antiviral signaling mediated by either the IFN-mediated JAK/STAT activation or NF-κB activation.

derly, and immunocompromised individuals and is associated with significant morbidity and mortality [10]. The increasing evidence has delineated the oncolytic features of RSV for apoptosis induction in various cell lines (Figure). In particular, its effects in human lung carcinoma cells of the A549 line are mediated via endoplasmic-reticulum-specific stress-activated caspase (caspase-12) [11, 12]. RSV has been shown to induce apoptosis in human prostate cancer cells including PC-3 through the down-regulation of NF-κB and stimulation of the intrinsic apoptosis pathway or the defect of the antiviral cytokine IFNα/β-induced STAT-1 activation [7, 13]. The studies on cervical cancer cells demonstrated that the oncolytic activity of RSV potentially resulted in apoptosis and

autophagy via TNF-α induction and ROS-BAX-mediated mechanisms [14]. An RSV-induced cytotoxic effect and apoptosis were also reported to occur in human skin cancer cells of the A431 line [8]. It has been elucidated that RSV fusion (F) protein, an integral membrane protein of the viral envelope, was responsible for the oncolytic effect of RSV in A549 cells. RSV F protein activates the caspase cascade pathway and causes p53 phosphorylation resulting in the activation of apoptosis [15].

The potential of RSV as an OV can be justified taking into account that it causes only asymptomatic infection in adults and is mildly pathogenic in infants and children. Moreover, RSV does not evoke a strong immune response in infected hosts, therefore, causing minimal damage to the host. Also, since the RSV genome is relatively small, it can be easily manipulated for desirable effects in the host using safe replication vectors [15, 16].

Oncolytic activity of RSV in prostate cancer cells. RSV has been shown to exert oncolytic activity in androgen-independent prostate tumor cells because of their defective NF-κB pathway [7, 13]. Since NF-κB is a critical factor in antiviral response against RSV, the defective NF-κB activation supports the oncolytic activity of RSV. Echchgadda [7] demonstrated that the enhanced infectivity of RSV in PC-3 cells results in caspase-3-mediated apoptotic death of prostate cancer cells *in vitro* and *in vivo* in immune-deficient nude mice. Besides, RSV induces tumor necrosis factor-α (TNF) produced in RSV-infected cells, which results in tumor cell apoptosis. Furthermore, it has been shown that RSV can induce apoptosis in androgen-independent human prostate cancer PC-3 cells by down-regulation of NF-κB and stimulation of the intrinsic apoptotic pathway. Besides, RSV induces apoptosis in androgen-sensitive and androgen-refractory prostate cancer cells by utilizing the deficiency in the antiviral signaling mediated by either the IFN-mediated JAK/STAT activation or NF-kB activation in virus-infected cells.

It was also shown that in androgen-dependent LNCaP prostate cancer cells, RSV infectivity and virus-induced apoptotic death were much higher as compared to the RWPE-1 cell line originating from the normal prostate. The increased apoptosis of LNCaP cells was traced to the blockade of STAT-1 activation causing inhibition of IFN-dependent antiviral gene expression and enhanced RSV replication leading to apoptosis. This is in contrast to the RSV-mediated apoptosis induction in androgen-independent PC-3 cells due to inhibition of NF-κB-dependent antiviral genes. Altogether, these results suggest that the oncolytic activity of RSV can be harnessed for developing virotherapy of prostate cancer.

Oncolytic effect of RSV in the human skin cancer cell line. The role of RSV in the modulation of cell growth and apoptosis was also investigated in A431 cells. The results indicated that A431 cell growth was inhibited following infection by RSV (MOI of 3) 48 h post-infection accompanied by the induction of apoptosis [8]. This study highlights the relevance of RSV on the cell death pathways in skin malignancies. However, the intracellular targets for RSV-induced apoptosis in skin cancer cells have not been analyzed yet.

Oncolytic activity of RSV on hepatocellular carcinoma cell lines. Among the assayed hepatocellular carcinoma (HCC) cell lines, apoptosis was induced by RSV infection in Huh-7 cells only. However, no apoptosis after RSV infection in other HCC cell lines (BNL-HCC, Hep3B, and SNU-739) was observed [17]. Therefore, it can be postulated that the effect of RSV infection on cancer cell growth seems to vary depending on the type of infected cells.

Oncolytic activity of RSV on cervical cancer cell lines. RSV oncolytic activity on the TC-1 murine cell line producing HPV-16 E6/E7 resulted in apoptosis and autophagy induction, caspase-3 activation, ROS generation, and cell cycle inhibition [13]. The cytopathic effect of RSV decreased the TC-1 cancer cell viability significantly at MOI of 10 and 15. Annexin V/PI analysis for determining the rate of cellular apoptosis induced by RSV showed that such apoptosis induction was MOI-dependent. The effect of oncolytic RSV on the intracellular reactive oxygen species (ROS) production demonstrated that RSV-infected TC-1 cells produced more ROS compared to the control cells in an MOI-dependent manner.

RSV-mediated apoptosis of lung cancer cells. Several studies reported that primary RSV induces apoptosis in A549 cells [11, 12, 16, 18]. It has been demonstrated that RSV F protein is responsible for the oncolytic effect of RSV in these cells and is a strong initiator of apoptosis. It triggers caspase cascade, causes p53 phosphorylation with activa-

tion of its transcriptional activity, and conformational activation of pro-apoptotic BAX [15]. The results demonstrated that RSV induces apoptosis through tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) pathway. Moreover, RSV infection also induced several pro-apoptotic factors of the BCL-2 family and caspases-3, -6, -7, -8, -9, and -10, constituting the death receptor- and mitochondrion-dependent apoptotic pathways. RSV also strongly induced anti-apoptotic factors of the BCL-2 family, especially Mcl-1. This might account for the delayed induction of apoptosis in RSV-infected cells in the absence of exogenous induction of the TRAIL pathway [16].

Conclusions and perspectives. The limitations of conventional cancer therapies have pushed the field of oncology toward exploring other novel immunotherapeutic strategies to inhibit cancer growth. Oncolytic virotherapy is one such promising strategy with the potential to improve cancer therapeutics with a multitude of the OVs is at various stages of clinical development. One of the major advantages of the OVs is the possibility to genetically engineer them for favorable host's outcomes. Despite the progress in OV therapy, there are limitations and further challenges, such as delivering the OVs to the targeted tumor sites, especially solid tumors, and the presence of neutralizing antibodies which limit its efficacy. However, the efficacy of OV therapy can be further improved via combination with other immunotherapy regimens or arming OV with the functional transgenes, a novel combination of OVs can be developed according to the type and stage of cancer. Therefore, combination approaches promise an increase in therapeutic efficacy offering personalized solutions for cancer patients. Currently, several Phase I and II clinical trials using OVs have been completed or are ongoing for treating different cancer types. Studies analyzing the possible role of RSV in the oncolysis of different types of cancer cells have highlighted its oncolytic potential. Even though the studies conducted with RSV oncolytic virotherapy are limited at present, engineering of RSV with other immunostimulatory substances can improve the usefulness of RSV-mediated oncolytic virotherapy, they are scarce so far. Future studies are necessary to unravel the molecular mechanisms involved in the RSV-mediated oncolytic effects and its potential application in oncolytic therapy.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Authors' contributions

DD, IMA, GAD, and RB wrote the manuscript. All authors read, edited, and approved the manuscript.

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Д. Дахризал [1], І.М. Азіз [2], Г.А. Дудін [1], Р. Бхат [2]
[1] Політехнічний коледж, Бенгкулу, Індонезія
[2] Політехнічний коледж, університет короля Сауда, Ріяд, Саудівська Аравія

ОНКОЛІТИЧНА АКТИВНІСТЬ РЕСПІРАТОРНО-СИНЦИТІАЛЬНОГО ВІРУСУ ЛЮДИНИ

Онколітичні віруси — це нові перспективні засоби лікування раку. Завдяки онколітичній віротерапії можна досягти специфічної загибелі пухлинних клітин та імунної костимуляції, що примножує протипухлинну імунну відповідь організму. Завдяки методам генної інженерії можлива також експресія онколітичними вірусами різних імуностимуляторних молекул для оптимізації та підсилення онколітичної віротерапії. Ефективність онколітичної віротерапії показана в багатьох доклінічних дослідженнях на різних пухлинах, що дозволяє досягнути персоналізованої терапії онкологічних захворювань. Респіраторно-синцитіальний вірус, РНК-вмісний вірус родини *Pneumoviridae*, спричинює інфекції нижніх відділів дихальних шляхів у немовлят та імуноскомпрометованих дорослих. Онколітична активність цього вірусу, що показана в клітинах раку передміхурової залози, печінково-клітинного раку та раку шкіри, опосередковується переважно через активацію апоптотичної клітинної смерті, що асоціюється з порушенням активації NF-κB або дефектністю активації STAT-1, індукованої інтерфероном α/β. Дослідження, проведені з клітинами раку шийки матки, показали, що інфікування респіраторно-синцитіальним вірусом приводить до активації автофагії та апоптозу, що опосередковується сигнальними шляхами ROS-BAX та TNF-α. Раціональне сполучення онколітичної віротерапії з іншими засобами терапії може бути корисним в тих випадках, коли відповідь на загальноприйняті засоби терапії є обмеженою. Стаття присвячена обговоренню підходів до онколітичної віротерапії із застосуванням респіраторно-синцитіального вірусу.

Ключові слова: онколітична віротерапія, онколітичні віруси, імунотерапія, респіраторно-синцитіальний вірус.