

Review Article

A Short Synopsis of the Current Status of Double-Stranded RNA-Mediated Immunokilling for Gastrointestinal Cancer

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Abbreviations

ASC: Apoptosis-associated speck-like protein containing a caspase-activating and recruitment domain; dsRNA: double-stranded RNA; IFN: interferon; IL: interleukin; IRF: Interferon Regulatory Factor; MAVS: Mitochondrial Antiviral Signaling adaptor; MDA-5: Melanoma Differentiation-Associated gene 5; NF- κ B: Nuclear Factor- κ B; NLRP: nucleotide-binding domain and leucine-rich repeat containing gene family pyrin domain; RIG-I: retinoic acid-inducible gene-I; RLR: retinoic acid-inducible gene-I-like receptor; TLR: Toll-like receptor; TRIF: Toll/interleukin-1 receptor domain-containing adaptor inducing interferon- β .

Introduction

In 1891, William B. Coley documented malignancy regression after erysipelas infection and based on this observation, initiated treatment of an unresectable tumor by inoculation with Streptococcal cultures in a patient. The tumor regressed and the individual was disease-free for 8 years [1]. He then combined heat-inactivated *Streptococci* and *Serratia marcescens* in a vaccine to treat patients with malignancies and this was eventually called Coley's toxin [2]. The mechanism of action was unknown until the discovery of Toll-Like Receptors (TLRs) in human in late 1990s [4], and it is now known that activation of TLRs induces an immune mechanism that destroys cancer cells.

Interaction between the Immune System and Cancer

The intellectual perspective of the interaction between host immune system and transformed cell has undergone evolution from the earlier concept of immune surveillance [4] to the current cancer immune editing theory [5]. In the latest iteration, recognition of cancer antigen by immune cell to elicit potent tumor-specific Cytotoxic T Lymphocyte (CTL) response is believed to be the core of cancer immune therapy [6]. CTLs migrate to the tumor region and are activated by recognition of the tumor-associated antigen and the MHC complex. The activated tumor-specific CTLs attack the tumor

Abstract

Significant advances in cancer immunotherapy have been made in recent years and one such tool is the use of double-stranded RNA (dsRNA) which is the molecular pattern of virus infection which are recognized by pattern recognition receptors. The combination of dsRNA and its receptors induce a significant immune response and trigger cancer cell apoptosis in vivo. In this paper, the use of dsRNA in immunotherapy of gastrointestinal cancer is briefly reviewed.

Keywords: Double-stranded RNA; Gastric cancer; Colorectal cancer; Immunotherapy; Toll-like receptor 3

cells by releasing perforins or engaging Fas/FasL to induce apoptosis of tumor cells and regression of the tumor [7].

Signals Triggered by dsRNA

Double-stranded RNA (dsRNA) is the ligand of TLR3. Upon sensing dsRNA, TLR3 translocates from endoplasmic reticulum to endosome [8] and activates signal pathways, e.g. the nuclear factor- κ B (NF- κ B) pathway to induce pro-inflammatory cytokines [9], the Interferon Regulatory Factor (IRF) 3/7 pathway to induce type I interferon [10]. The unique pathway of dsRNA-TLR3 interaction leads to activation of Fas-Associated cell Death Domain (FADD) protein-caspase-8 axis to initiate cell apoptosis [11]. This property is of importance in cancer immunotherapy. dsRNA can also activate retinoic acid-inducible gene-I (RIG-I)-like receptors (RLRs) including RIG-I and melanoma differentiation-associated gene 5 (MDA-5) to induce type I interferon production [12]. In addition, dsRNA is able to associate with nucleotide-binding domain and leucine-rich repeat containing gene family pyrin domain 3 (NLRP3) to induce inflammasome and IL-1 and IL-18 production which elicits an immune response [13] (Figure. 1). Other dsRNA sensors also exist and play different roles on host immune response [14]. A recent review described these signal pathways in detail [14]. It appears that activation of TLR3 rather than MDA-5 is crucial for dsRNA mediated cancer immunotherapy [15].

TLR is Involved in Cancer Immunotherapy

Current evidence has documented that TLR as a subclass of pattern recognition receptor (PRR) actively participates in innate and adaptive immune responses of the host to eliminate invading pathogens and transformed cells [16]. A recent study suggested that endosomal TLRs like TLR3 and TLR9 are more effective in the induction of CTL response than cell surface TLRs [17]. TLRs are present not only in immune cells but also in cancer cells [18]. Thus, application of dsRNA in the immunotherapy of cancer may involve 2 mechanisms: activation of immune cells to trigger innate and adaptive immune response overcoming the immunosuppressive

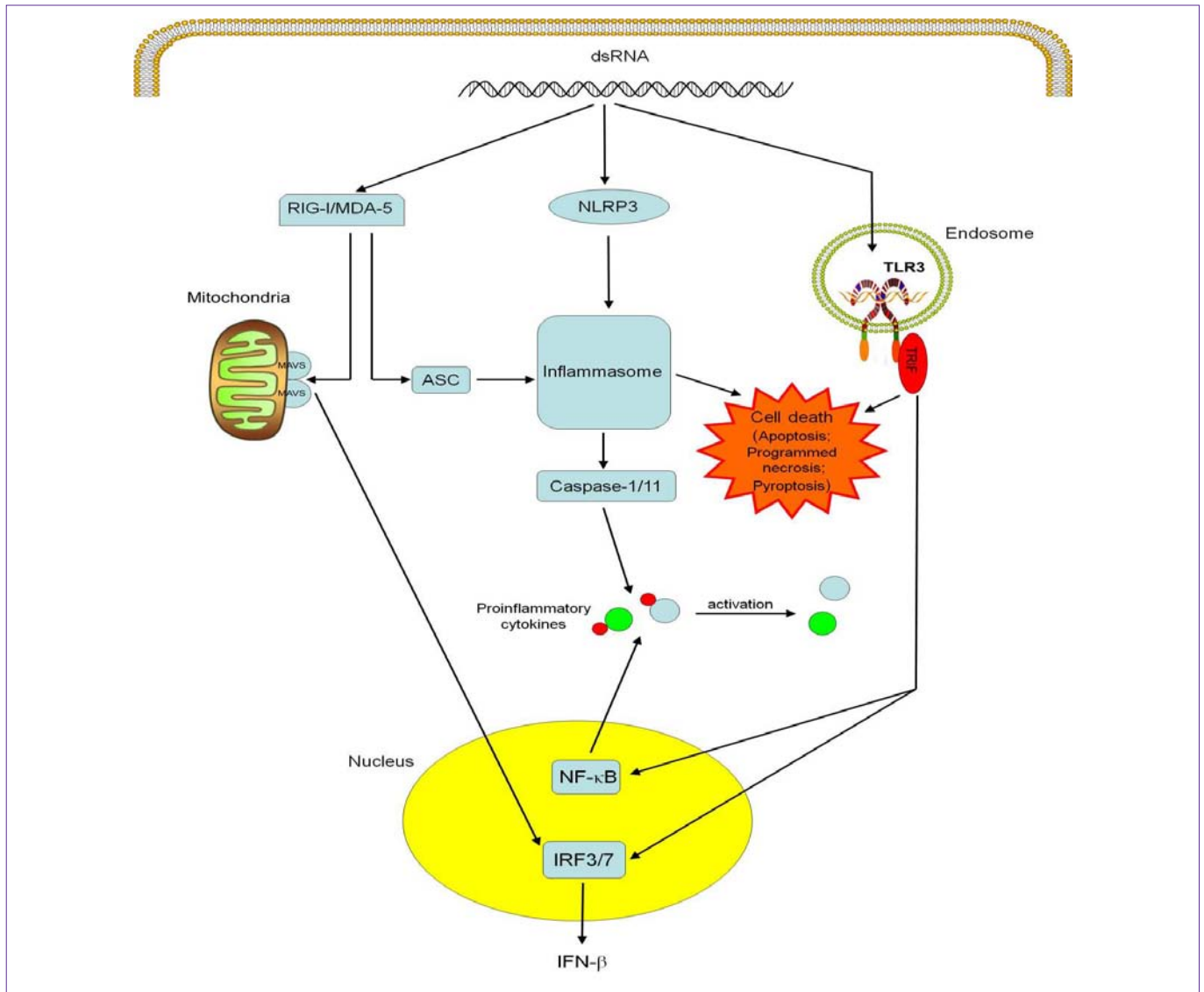


Figure 1: dsRNA induced signals. dsRNA can be recognized by endosomal TLR3. Activated TLR3 associates its ligand TRIF, and then activates either NF-κB or IRF3/7 pathways to induce production of proinflammatory cytokines or type I interferon. Activation of TLR3 is also able to trigger cell death through apoptosis or programmed necrosis. dsRNA can also associate RLRs, e.g. RIG-I or MDA-5. The activated RIG-I or MDA-5 combines its adaptor protein MAVS to activate IRF3 or IRF7 which induce type I interferon production. The activated RIG-I or MDA-5 can also associate ASC to induce assembly of inflammasome. Through combination of dsRNA with NLRP3, formation of inflammasome is induced which in turn promotes IL-1 and IL-18 production. The inflammasome can also induce cell death via pyroptosis.

microenvironment created by cancer cells, and direct action on the dsRNA receptors expressed in cancer cell to induce cellular apoptosis, programmed necrosis and perhaps pyroptosis resulting in tumor shrinkage. Polyinosinic-polycytidylic acid [poly (I:C)], a synthetic dsRNA analogue is able to convert tumor-supporting macrophages to tumoricidal effector cells in mice. This is mediated by TNF-α signaling that is dependent of TLR3-Toll/interleukin-1 receptor domain-containing adaptor inducing interferon-β (TRIF) pathway and is independent of myeloid differentiation factor 88 (MyD88) pathways and MDA-5-Mitochondrial Antiviral Signaling Adaptor (MAVS) pathway [19].

dsRNA Applied in Immunotherapy of Gastrointestinal Cancer

dsRNA have been used [20-28] but specifically for disease

related to the GI tract, the data is scant. Early studies showed that poly (I:C) can inhibit the growth of human colon carcinoma cells in vitro [29], and block the cytotoxicity of 5-fluorouracil by interfering with the bioactivation of the latter [30]. dsRNA analogues are able to synergistically enhance the cytotoxic effect of interferon γ on human colon carcinoma cell line [31]. They can also modulate the cancer microenvironment by killing TLR3-expressing cancer cells, inducing T cell and NK cell infiltration, and activating TLR3-expressing NK cells [32]. Activation of TLR3 in hepatocyte cancer cell line HepG2 results in cellular apoptosis rather than pro-inflammatory cytokine production [33]. Similar results were also found in gastric cancer cell lines BGC-823 cells and AGS through RLRs signaling [34]. Another study reported that over-expression of TLR3 was correlated with poor prognosis [35]. Yet the expression of TLR3 in this study, analyzed by a computerized image analysis system, did not distinguish the surface

and the endosomal expressions of TLR3 [35]. The surface TLR3 may activate different signal pathways and result in different biological effect in comparison with the endosomal TLR3 [36]. TLR3 expression has been recognized as a biomarker for the therapeutic efficacy of dsRNA in breast cancer [37]. Thus, the anti-cancer effect of dsRNA in different cancer may be mediated by distinct signal pathways. Further study has shown that the activation of intracellular TLR3 induces cell apoptosis while activation of cell surface TLR3 can activate NF- κ B activity but not affect cell viability [36]. dsRNA can also be used as an adjuvant to enhance the anti-cancer immunity of CEA-based vaccine in murine colorectal cancer model [38,39].

Currently, several synthetic dsRNA analogues have been tested or in clinical trials. Currently, a phase I/II clinical trial using poly (I:C) with substitution of every 13th cytosine (C) with uracil (U) [poly (I:C12U)], interferon and celecoxib in fighting recurrent colorectal cancer is being conducted [40]. Another randomized, double-blind, placebo-controlled trial in newly diagnosed advanced colon adenomatous polyps to evaluate the effect of poly (I:C) stabilized with poly L-lysine and carboxymethyl cellulose [poly (ICLC)] adjuvanted MUC1 peptide vaccine in preventing the recurrence of adenomatous polyps and preventing the development of colorectal cancer is also active [41]. A phase II trial using this poly (ICLC) with MUC1 vaccine in preventing adenomatous polyps advancing into colon cancer and preventing polyps from recurring has been completed early in 2014 [42].

Perspectives

Cancer immunotherapy as a therapeutic approach has received increasing attention [43] with dsRNA analogues being one tool of the anti-cancer armamentarium. They are pregnant with therapeutic opportunities either as possible adjuvants of cancer vaccine or as combination therapy for the treatment of patients with gastrointestinal cancer.

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