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# Sigma 1 Receptor plays a prominent role in IL-24-induced cancer-specific apoptosis

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ABSTRACT

Interleukin-24 (IL-24), a member of the IL-10 cytokine family, is an immunomodulatory cytokine that also displays broad cancer-specific suppressor effects. The tumor suppressor activities of IL-24 include inhibition of angiogenesis, sensitization to chemotherapy, and cancer-specific apoptosis. We show that Sigma 1 Receptor (S1R), a ligand-regulated protein chaperone contributes to IL-24 induction of apoptosis. IL-24 generated from an adenovirus expressing IL-24 (Ad.IL-24) induces cancer-specific apoptosis by inducing an endoplasmic reticulum (ER) stress, reactive oxygen species production, and calcium mobilization. The present studies reveals that S1R is required for Ad.IL-24-induced cell death. We provide several lines of evidence to confirm a physical and functional interaction between IL-24 and S1R including: (a) S1R and IL-24 co-localize, as judged by immunocytochemical analysis studies; (b) S1R and IL-24 co-immunoprecipitate using either S1R or IL-24 antibody; (c) S1R agonist (+)-SKF10047 inhibits apoptosis by Ad.IL-24; (d) (+)-SKF10047-mediated inhibition of Ad.IL-24 results in: diminished ER stress protein expression; (e) Calcium mobilization; and (f) ROS production. Collectively, these data demonstrate that S1R interacts with IL-24 and suggest that IL-24:S1R interaction determines apoptosis induction by Ad.IL-24. These studies define Sigma 1 Receptor as a key initial mediator of IL-24 induction of cancer-specific killing. These findings have important implications for our understanding of IL-24 as a tumor suppressor protein as well as an immune modulating cytokine.



## 1. Introduction

It has been shown that Ad.IL-24 (INGN 241) activity by intratumoral injection in patients with advanced solid tumors is safe and able to induce as much as 70% apoptosis in tumors after a single injection of recombinant virus and that multiple injections generated clinical responses [1]. Moreover, secreted IL-24 protein, generated from Ad.IL-24-infected cells, promotes antiangiogenic, immunostimulatory, radiosensitizing and "bystander" antitumor activities. IL-24 as a cancer gene therapeutic has several obvious advantages over other candidate molecules, including high tumor cell specificity, anti-angiogenic properties, ability to radiosensitize, as well as cause growth-suppressive effects that are independent of p53, p16, Rb and BAX mutational status [2]. In addition, it may be used in conjunction with existing p53-based therapeutics and has shown potential synergism with immuno- and chemo-therapy, potentially allowing a reduction in dose of all components in a given therapy [2]. However, despite the extensive studies, questions remain about how IL-24 exerts its tumor-specific effect.

Interleukin 24 (IL-24) is a member of the IL-10 family of cytokines. It binds to IL-20 receptor complexes and activates the STAT signaling cascade [3]. IL-24 possesses the properties of a classical cytokine as well as tumor suppressor protein [2]. When expressed at supraphysiological levels, by means of an adenoviral (Ad) expression system (Ad.IL-24), IL-24 induces growth suppression and apoptosis in a broad spectrum of human cancer cells, without exerting any deleterious effects on their normal counterparts [2]. Furthermore, secreted IL-24 protein generated from Ad.IL-24 infection, induce cancer-specific apoptosis [4]. Ad.IL-24 induces cancer-selective apoptosis even in the absence of JAK/STAT signaling [5]. As evidenced by the expression of ER stress markers (BiP, CHOP, and phospho-eIF2α) Ad.IL-24 or secreted IL-24 protein, induce ER stress [6,7]. Ad.IL-24 or secreted IL-24 protein also generates reactive oxygen species (ROS) in the mitochondria [8,9]. Ad.IL-24 induces ceramide production in cancer cells [10]. Adenovirus delivery of IL-24 inhibits β-catenin and phosphatidylinositol 3'-kinase signaling pathways in lung cancer cells and activates Fas–FasL signaling in ovarian cancer cells [11]. Infection of melanoma cells with Ad.IL-24 results in activation of death ligands (FasL), tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), and their respective death receptor signaling pathways [12].

We have shown that secreted IL-24 protein induces a robust expression of endogenous IL-24 and subsequent induction of

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tumor-specific killing through an ER stress-mediated pathway as well as by ROS production [4]. We have shown that IL-24 protein induces stabilization of its own mRNA without activating its promoter [4]. We have shown that Ad.IL-24 induces $p38^{MAPK}$. Consistent with these reports, Otkjaer and colleagues have recently shown that $p38^{MAPK}$ regulates IL-24 expression by stabilization of the 3'UTR of their mRNA. In recent years there a consensus has arisen that the ER stress pathway is the initial pathway in IL-24-induced apoptosis [13]. Recent studies further supported that IL-24 causes ER stress by physically interacting with the ER chaperon protein BiP [14]. The authors explain that IL-24:BiP binding might be mediated by the interaction of IL-24 with as yet unidentified protein that confers cancer cell specificity [14]. The precise molecules mediating this pathway remain unclear.

Sigma 1 Receptor (S1R) is a ligand-regulated protein chaperone subject of an evolving research area that could lead to therapeutic developments for many diseases [15]. S1R has also been shown to modulate endothelial cell proliferation and can control angiogenesis, which makes it a promising target for oncology applications [16]. S1R was recently identified as a receptor chaperone whose activity can be activated/deactivated by specific ligands. Manipulation of S1R can yield either cytoprotective or cytotoxic actions. The stimulation with sigma "agonists" induces S1R dissociation from BiP and S1R delocalization, while sigma ligands classified as "antagonists" impede this process [17–19]. S1R agonists promote cellular survival by preventing oxidative stress caused by ischemia, diabetes, inflammation, and amyloid toxicity [20]. Conversely, antagonists of the S1R inhibit tumor cell survival and induce apoptosis [17]. Sigma antagonist-mediated cell death is inhibited by the prototypic sigma-1 agonists (+)-SKF10047 [21]. Furthermore, systemic administration of sigma antagonists significantly inhibits the growth of mammary carcinoma xenografts, prostate tumors, and lung carcinoma in the absence of side effects [22]. On the other hand, several normal cell types such as fibroblasts, epithelial cells, and even sigma receptor-rich neurons are resistant to the apoptotic effects of sigma antagonists [22]. Cellular susceptibility appears to correlate with differences in S1R coupling rather than levels of expression. In cancer cells only, sigma antagonists evoke a rapid rise in cytosolic calcium that is inhibited by S1R agonists. In tumor cells, sigma antagonists cause activation of phospholipase C and concomitant inhibition of phosphatidylinositol 3'-kinase pathway signaling [23].

In this report, we document for the first time that S1R plays a decisive role in IL-24-mediated apoptosis. We provide several lines of evidence to confirm a physical and functional interaction between IL-24 and S1R. These studies define Sigma 1 Receptor as a key initial mediator of IL-24 induction of cancer-specific killing. These findings have important implications for our understanding of IL-24 as a tumor suppressor protein as well as an immune modulating cytokine.

## 2. Materials and methods

### 2.1. Virus infection

The IL-24 expressing replication defective Ad.IL-24 and corresponding empty adenovirus vector lacking exogenous gene, used as a control (Ad.vector) were custom engineered by Vector Biolabs, Inc. (Philadelphia, PA).

### 2.2. Cells and culture conditions

RWPE1, LNCaP, DU145, and PC3 (ATCC, Rockville, MD) cell lines were grown in DMEM with 10% fetal bovine serum (FBS) 1% penicillin/streptomycin. All cell lines were cultured in humidified atmosphere at 37 °C with 5% $CO_2$ and media was replaced every alternate day. (+)-SKF10047 was purchased from Tocris (Tocris, UK).

### 2.3. Western blot analysis

Protein extracts were prepared with RIPA buffer containing a mixture of protease inhibitors as described [10]. Fifty micrograms of protein was applied to a 12% SDS/PAGE and transferred to nitrocellulose membranes. The membranes were probed with polyclonal or monoclonal antibodies to IL-24, p-eIF2α, BiP, CHOP, Sigma 1 Receptor, and β-actin.

### 2.4. MTT assays

Cells were plated in 96-well dishes ($1 \times 10^3$ cells/well) in DMEM containing 10% FBS and allowed to attach for 12 h prior to treatment(s). Inhibitors were added 4 h after infection with adenovirus. Cell growth and viable cell numbers were monitored by 3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) staining as described [10].

### 2.5. Annexin V binding assays

Cells were trypsinized, washed once with complete medium and PBS, resuspended in 0.5 ml of binding buffer containing 2.5 mmol/L $CaCl_2$, and stained with allophycocyanin-labeled Annexin V (Becton Dickinson Biosciences, Palo Alto, CA) and propidium iodide (PI) for 15 min at room temperature. Flow cytometry assays were performed as described [10].

### 2.6. Colony formation assays

Cells were infected with 100 pfu/cell with Ad.vector or Ad.IL-24. The next day, 200–500 cells were seeded to determine colony-forming ability. After 2 weeks of incubation, colonies were fixed, stained with 5% Giemsa solution, and colonies of >50 cells were enumerated as described [10].

### 2.7. Immunofluorescence

Cells were seeded onto chamber slides (Falcon; BD Biosciences, San Jose, CA) and maintained in DMEM with 10% fetal bovine calf serum, 24 h postinfection, cells were fixed with 2% paraformaldehyde, permeabilized by 0.1% Triton X-100, and then incubated with primary antibodies: IL-24, and S1R. Controls were incubated with only the secondary antibodies under the same experimental conditions.

### 2.8. Co-immunoprecipitation of S1R with IL-24

Cells were infected with Ad.vector or Ad.IL-24. After 48 h, protein was extracted from subconfluent cultures using lysis buffer (Pierce, Rockford, IL) containing 1 mM phenylmethlsulfonylfluoride (Sigma–Aldrich, Inc.) and quantified using the BCA protein assay kit (Pierce, Rockford, IL). Antibodies were conjugated to Protein-G beads according to the Sigma Protein-G Immunoprecipitation Kit manufacturer's instructions (Sigma–Aldrich, Inc.). Western blot analysis was done as described before using the following primary antibodies at 1:1000 dilutions: anti-IL-24, and anti-S1R. Secondary antibodies specific for heavy chain of immunoglobulin G (IgG) were used as the light chain of IgG interfered with detection of IL-24 because of similar size.

### 2.9. Calcium imaging

For calcium ($Ca^{++}$) imaging, cells were plated in 35 mm glass bottom petri dishes (MatTek) and allowed to attach for 12 h prior to treatment(s). Inhibitors were added 4 h after infection with adenovirus. After 12 h, cells were then rinsed with a Ringer's solution maintained at 37 °C. Cells were then incubated in Ringer's solution containing 0.5 μM Fura-2 tetra-acetoxymethyl ester (Fura-2) (molecular probes), 10% Pluronic F127 and 250 μm sulfinpyrazone (Sigma–Aldrich, Inc.) for 40 min at 22 °C. Fura-2 was excited by alternating 340 and 380 nm light and images were obtained every 50 ms as a measure of $Ca^{++}$ concentration. Background intensity was zero. A bolus injection brought the stimulant concentration in the cell bath to either 1 mM glutamate (Sigma–Aldrich, Inc.) or 1 mM N-methyl-D-aspartic acid plus the co-stimulator 1 mM glycine. Prism Software (GraphPad Software Inc version 6.0C) was used to analyze the results. Intra-group analysis was done with ordinary one-way ANOVA to compare the mean of raw calcium ratios of each treatment group with a control group. A Dunnet's multiple comparison test with a single pooled variance was also performed on the 4 treatment groups. A significance of 0.01 was used in the analysis.

### 2.10. Assessment of reactive oxygen species (ROS) generation

DU-145 cells were seeded in 96-well plates at a concentration of $1 \times 10^4$ cells/well and were infected with Ad.IL-24 for 12 h. The cell cultures were treated with 10 μM 2,7-dichloro-fluorescein diacetate (DCFH-DA; Sigma–Aldrich, St. Louis, MO) in PBS for 30 min. After incubation, the media was discarded, and the cells were washed with PBS. The fluorescence intensity was determined using a fluorescence plate reader at 485 nm for excitation and 530 nm for emission.

## 3. Results

### 3.1. Treatment of cells with Sigma 1 Receptor (S1R) agonist prevent IL-24 killing

Given the similarities between the effects of antagonists of S1R and IL-24 on specific signaling pathways, we addressed the questions of whether IL-24 could trigger S1R-dependent apoptosis. We hypothesized that IL-24 acts as a S1R antagonist in mediating tumor cell death. To test this hypothesis Ad.IL-24 was used to treat normal human immortalized epithelial cells (RWPE1), and three metastatic prostate cancer cell lines (LNCaP, DU145 and PC-3) in the presence or absence of the specific S1R agonist, (+)SKF-10047, and measured cell viability and induction of apoptosis by MTT, clonogenic, and Annexin V-FITC/PI assays. (+)SKF-10047 inhibited Ad.IL-24-mediated killing in PC-3, LNCaP and DU145 cells. Ad.IL-24 had only a slight effect on viability, clonogenic capacity, or apoptosis of normal RWPE1 cells (Fig. 1A–C). Taken together, these data suggest that IL-24-induced cell death in cancer cells would be achieved by antagonizing S1R, and is therefore inhibited by an S1R agonist.

### 3.2. Sigma 1 Receptor is critically involved in Ad.IL-24-induced ER stress, ROS production, caspases-3 activation, and calcium mobilization

We reported that after Ad.IL-24 infection IL-24 protein localized in the ER and induces ER stress resembling an UPR [6,7]. We show here that up-regulation by IL-24 of several ER stress markers, including p-eIF2α, CHOP, and BiP, were inhibited by treatment with (+)SKF-10047 (Fig. 2A). Prior studies examining the effects of IL-24 in primary human glioblastoma cells have shown calcium ($Ca^{++}$) elevation [24]. We determined whether Ad.IL-24 caused any changes in the cytosolic levels of $Ca^{++}$ in prostate cancer cells and if $Ca^{++}$ mobilization is S1R-dependent after Ad.IL-24 infection. Ad.IL-24 infection increased cytosolic $Ca^{++}$ levels in prostate DU145 within 12 h (Fig. 2B). The increase in $Ca^{++}$ was blocked by S1R agonist (+)-SKF10047 (Fig. 2B). We determined the time course of mitochondrial changes (ROS generation) after treatment of DU145 cells with Ad.IL-24. Cells were infected with Ad.IL-24, collected at 24 h, and stained for ROS production with dichlorofluorescin diacetate (DCFH-DA). Fig. 2C shows that Ad.IL-24 increased ROS, were inhibited by treatment with (+)SKF-10047 (Fig. 2C). Taken together, the inhibition by an S1R agonist of IL-24-mediated ER stress, $Ca^{++}$ mobilization and ROS production, further strengthen the hypothesis that IL-24 action in cancer cells is mediated by an antagonistic effect of IL-24 on S1R.

### 3.3. Endoplasmic reticulum chaperone S1R interacts with IL-24

Comparative co-localization of IL-24 and S1R proteins was analyzed in DU145 cells after infection with the Ad.IL-24 virus. Comparison of the immunofluorescence data using different cells and secondary antibodies performed at independent times, yielded similar reproducible patterns of staining; representative data are presented for DU145 in Fig. 3A, demonstrating that IL-24 co-localized with S1R (Fig. 3A). The previous results led us to examine the possibility that S1R may interact with IL-24. Infection with Ad.IL-24 followed by immunoprecipitation using anti-S1R antibody and immunoblotting with anti-IL-24 antibody confirmed a physical interaction between these molecules (Fig. 3B). Experiments were also done in a reverse direction, immunoprecipitation was done using anti-IL-24 antibody and the membrane was probed with the anti-S1R antibody (Fig. 3C). IL-24 protein coimmunoprecipitated with S1R, demonstrating a physical interaction between these two molecules, converging with the above results in supporting the hypothesis that IL-24 could antagonize S1R.

## 4. Discussion

Defining the biochemical basis of cancer-selective activity of IL-24 provides an important entry point for rationally devising combinatorial approaches to enhance the therapeutic impact of this intriguing multifunctional antitumor molecule. IL-24 displays a broad range of antitumor properties including cancer-specific induction of apoptosis, inhibition of tumor angiogenesis, and modulation of anti-tumor immune responses [2]. The results presented here identify S1R as a key mediator of IL-24 induction of cancer-specific killing. S1R agonist (+) SK-10047 blocks Ad.IL-24-mediated cancer-selective apoptosis in prostate cancer cells (Fig. 1). Here we also report that ER stress response, ROS production, and calcium mobilization triggered after Ad.IL-24 infection is mediated through a S1R-dependent pathway (Fig. 2). Co-immunoprecipitation and co-localization studies revealed for the first time that IL-24 interacts with S1R (Fig. 3). Our results provide very strong evidence that Ad.IL-24 induces apoptosis through a S1R antagonistic mechanism. Taken together, our results indicate that IL-24 exerts a tumor-selective, ER stress, ROS production, calcium mobilization effect by acting through a S1R antagonistic mechanism. Our results, outlined in the model in Fig. 4, clearly reveal that IL-24 induces growth inhibition and apoptosis through a S1R-dependent pathway. These findings have important implications for our understanding of IL-24 as a tumor suppressor protein as well as an immune modulating cytokine.

We have demonstrated that IL-24 induces ER stress and this response could be the common upstream event [6,7]. Downstream targets of IL-24 after induction of ER stress include $p38^{MAPK}$, Calcium mobilization, ROS, and ceramide production

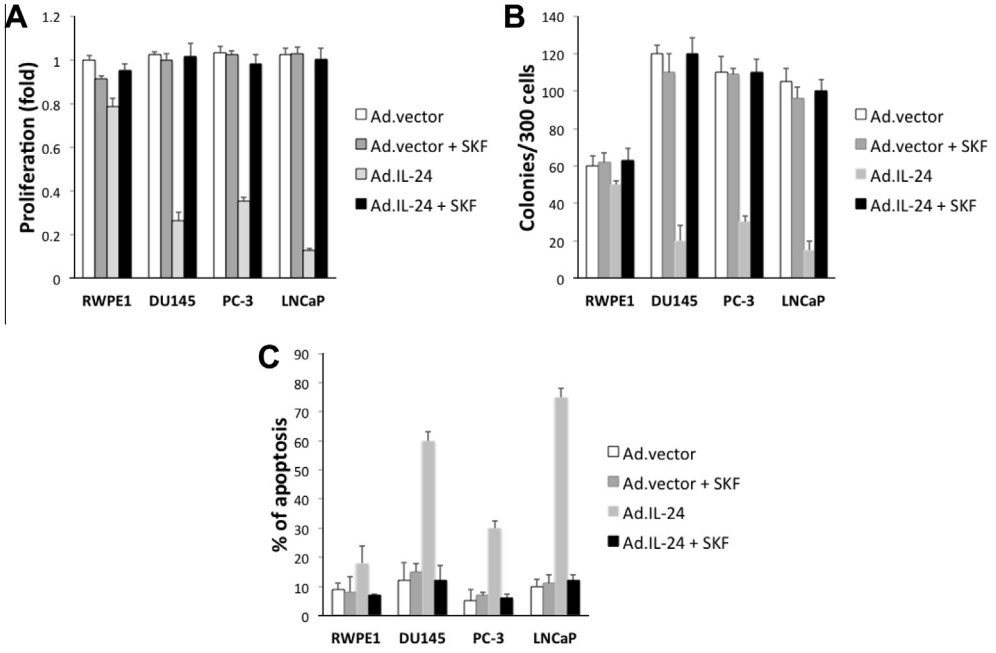


**Fig. 1.** Treatment of cells with Sigma 1 Receptor (S1R) agonist prevents IL-24-induced apoptosis. (A) Cells were infected with 100 pfu/cell of Ad.vector or Ad.IL-24, and treated with or without 10 μM (+)-SKF10047. Cell viability was determined by MTT assay 4 days post-infection. MTT absorbance of untreated control cells was set at 1 to determine relative number of viable cells. (B) Cells were incubated in the absence or presence of 10 μM (+)-SKF10047 after infection with Ad.IL-24. Forty-eight hours post-infection, percentage of apoptosis was determined by staining with Annexin V-FITC/PI. (C) Cells were incubated in the absence or presence of 10 μM (+)-SKF10047 after infection with Ad.IL-24. Cells were subjected to clonogenic assay for 2 weeks. Results shown are an average of three independent experiments ± SD.

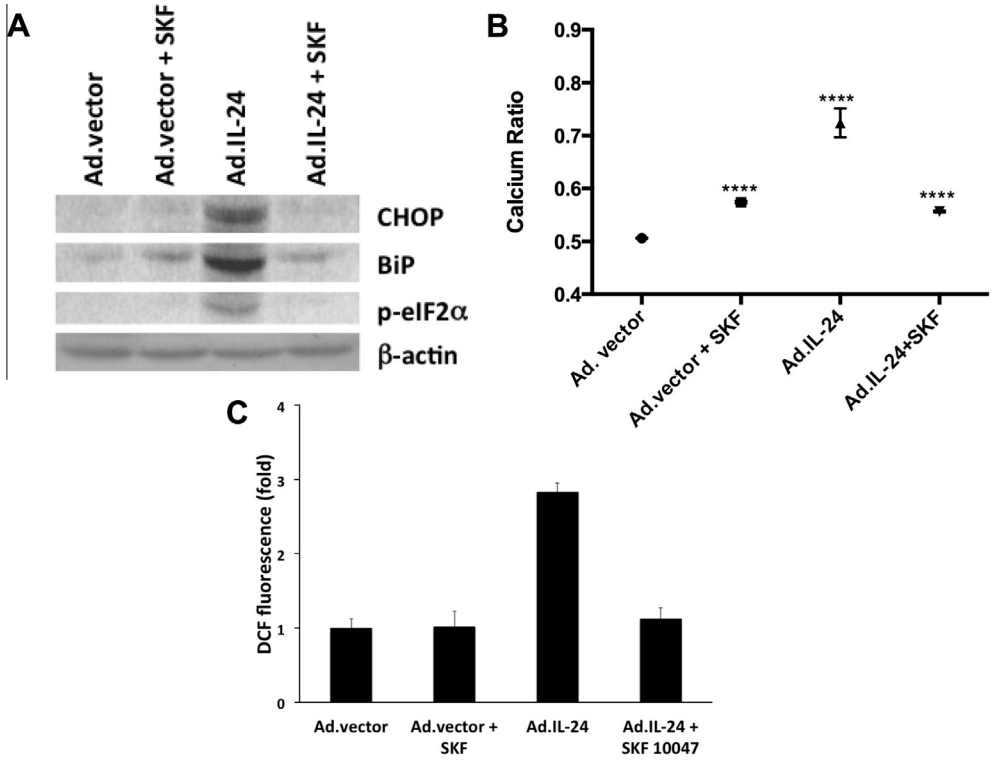


**Fig. 2.** S1R is critically involved in Ad.IL-24-induced ER stress, calcium mobilization, and ROS production. Cells were infected with 100 pfu/cell of Ad.vector or Ad.IL-24, and treated with or without 10 μM (+)-SKF10047 (SKF) for indicated times. (A) Changes in BiP, CHOP, and p-eIF2α proteins were evaluated by Western blot analysis 48 h after indicated treatments. (B) Cells were infected with 100 pfu/cell of Ad.vector or Ad.IL-24, and treated with or without 10 μM (+)-SKF10047. Twelve hours after infection, levels of cytosolic $Ca^{++}$ were measured. (C) DU-145 cells were infected with Ad.vector or with Ad.IL-24 and treated with or without 10 μM (+)SKF-10047 for 24 h. Intracellular ROS levels were measured with 10 μM DCF-DA 30 min after treatments. The results are expressed as the mean ± SD of three independent experiments.

[2]. Interestingly, Gupta et al. have documented that IL-24 directly interacts with the ER resident chaperone BiP [14]. The authors elegantly proved, with an extensive set of constructs, that IL-24:BIP interaction might be mediated by the interaction of IL-24 with as yet unidentified protein that confer cancer cell specificity [14]. Although efforts were made to identify interacting partner/s by

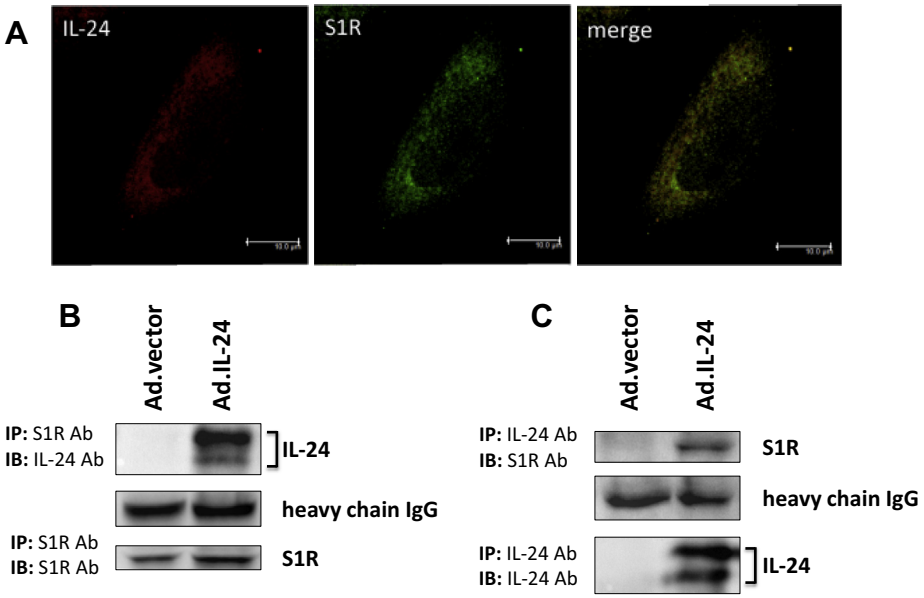


**Fig. 3.** IL-24 protein co-localizes with and binds to S1R. (A) DU-145 cells were infected with Ad.IL-24. After 24 h, cells were fixed and IL-24 and S1R proteins were detected by immunofluorescence using anti-IL-24 and anti-S1R antibodies. The analysis of co-localization of IL-24 and S1R was performed using a DMI6000B inverted confocal microscope with TCS SP5 system (Leica Microsystems CMS). (B) DU145 cells were infected with 100 pfu/cell of Ad.vector or Ad.IL-24 and immunoprecipitation analysis was done 48 h later using S1R antibody. (C) DU-145 cells were infected with 100 pfu/cell of of Ad.vector or Ad.IL-24 and immunoprecipitation analysis was done 48 h later using IL-24 antibody.

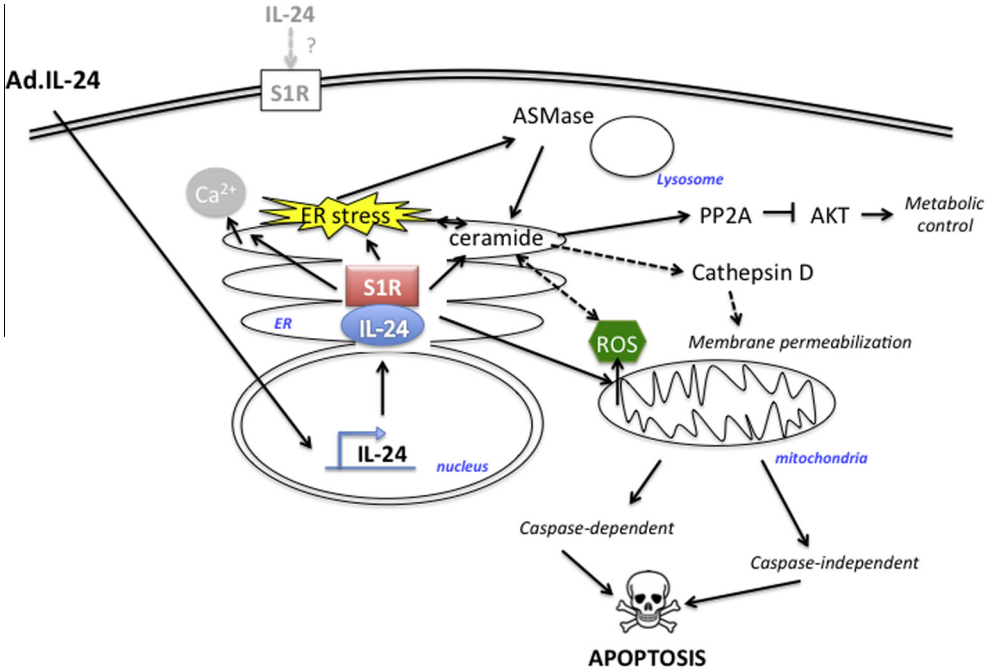


**Fig. 4.** Proposed model of Ad.IL-24 cancer cell-selective apoptosis induction. *Abbreviations:* IL-24, Interleukin 24; S1R, Sigma 1 Receptor; ROS, reactive oxygen species; $Ca^{++}$, calcium; ER, endoplasmic reticulum; PP2A, protein phosphatase 2A; ASMase, acid sphingomyelinase.

the yeast two hybrid screening approach, no clones were identified that could potentially interact with IL-24 [2]. We have demonstrated that Ad.IL-24 induces ceramide production, and that plays a key role in ROS production, which in turn, can generate additional molecules of ceramide [10]. We also have demonstrated that IL-24 protein generates additional molecules of IL-24 that induce more ER-stress culminating in an untenable imbalance resulting in apoptosis in cancer cells [4].

An important question, which remained unresolved, is why IL-24 has the abilities to selectively induce apoptosis in a large spectrum of human cancer-derived cell lines without harming normal cells. One possible reason for this differential killing effect involves inherent biochemical differences between normal and cancer cells (ER stress, ROS production and ceramide), another possibility is that IL-24 is able to target a molecule that only triggers apoptosis in cancer cells. The third option for this differential killing effect is that both of the above hypotheses are correct. The molecular properties of IL-24 and associated pathways will need to be analyzed in much greater detail to fully understand this cytokine. We therefore searched for a candidate molecule that

can consolidate the entire signal transduction pathway triggered by IL-24. In particular, it is postulated that IL-24 not only triggers ER stress and its downstream molecules (ROS, ceramide, calcium mobilization) but that IL-24 can also regulate gene products involved in invasion (β-catenin, FAK, and PI3K/PKB), angiogenesis (VEGF and TGF-β), and metastasis (β-integrin) [2]. Notably, IL-24 activates TRAIL, and Fas-FasL [11]. This is remarkably similar to the effects reported for S1R antagonists [15,17,19,22,23]. Furthermore, a recent study identified S1R binds to BiP and regulates ER stress, calcium mobilization, ROS and ceramide production [15,16,19].

Secreted IL-24 protein, generated from Ad.IL-24-infected cells, promotes antiangiogenic, immunostimulatory, radiosensitizing and "bystander" antitumor activities. IL-24 stimulates the immune system to generate secondary cytokines, such as TNF-α, IFN-γ, and IL-1 that evokes an antitumor immune response [2]. Secreted IL-24 protein, generated from Ad.IL-24-infected cells, exerts antiangiogenic activity by inhibiting endothelial cell differentiation and by blocking the activities of VEGF and TGF-α via inhibition of *src* activity within tumor cells [25]. We have demonstrated that IL-24 protein generates additional molecules of IL-24 that induces more ER-stress culminating in an untenable imbalance resulting in apoptosis in cancer cells [4]. Specifically, exogenous IL-24 protein induces growth inhibition and apoptosis only in cancer cells through a mechanism identical to Ad.IL-24 infection [4]. These observations coupled with our present findings suggest that IL-24-mediated IL-24 induction could involve an S1R-mediated mechanism as an event down-stream of IL-20 receptor activation by extracellular IL-24. As discussed in the present work these findings have important implications for our understanding of IL-24 as a tumor suppressor protein as well as an immune modulating cytokine.

In accordance with what has been observed with IL-24, the combination of immunosuppression, along with anti-inflammatory properties makes S1R ligands attractive molecules for therapeutic applications such as autoimmune diseases in which both immune and inflammatory disorders are involved [22]. Interestingly, S1R is known to translocate and remodel the plasma membrane [26]. Accumulating evidence indicate that S1R is overexpressed in many cancer cell lines, and contributes to the invasion and metastasis in many human tumors [23]. Aydar et al. have demonstrated that in breast cancer cells, S1R is associated with β1 integrin in lipid cholesterol-enriched rafts [26]. In response to environmental conditions encountered in cancer tissue, S1R may dynamically trigger various adaptation mechanisms, the nature of which being tightly dependent on the client protein available in a given tumor cell type. The discovery of surface localization of BiP as well as S1R in cancer cells reveals potential novel function, interaction with cell-surface receptors, and possible therapeutic implications. Therefore, it is tempting to speculate that IL-24 protein binds to S1R in the plasma membrane, a possibility we propose to address.

The results we present here support the hypothesis that Sigma 1 Receptor (S1R) may be the upstream initial signal transduction molecule common to these cascades of events involving IL-24-induced ER-stress dependent and independent downstream pathways. In summary, the identification of S1R as a key initial mediator of IL-24-cancer-specific apoptosis significantly broadens their therapeutic potential for tumors as well as provides new important knowledge for our understanding of IL-24 as an immune modulating cytokine.

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