Wuhan University
Journal of Natural Sciences

2016, Vol.21 No.6, 519-530

Article ID 1007-1202(2016)06-0519-12
DOI 10.1007/s11859-016-1205-2

Review

Apoptotic Signal Pathways and Regulatory Mechanisms of Cancer Cells Induced by IL-24

□ **LIU Huilin[1], CHEN Jiaojiao[1], JIANG Xingyan[1], WANG Tao[1], XIE Xiaoyuan[2], HU Hui[1], YU Fang[1], WANG Xingzhi[1], FAN Handong[1, 3†]**

1. Institute of Aging Research, School of Medicine, Hangzhou Normal University, Hangzhou 310036, Zhejiang, China;

2. The Third Affiliated Hospital, Sun Yat-Sen University, Guangzhou 510630, Guangdong, China;

3. Institute of Microbiology, Jiangxi Academy of Sciences, Nanchang 330029, Jiangxi, China

Abstract: The melanoma differentiation-associated gene-7(mda-7), IL-24, has the specific functions that induce cancer cell apoptosis without doing harm to normal cells. We systematically review the apoptotic signal pathways and their regulatory mechanisms induced by Ad.IL-24 and IL-24 in diverse cancer cells. IL-24 can participate in varied signal transduction pathways, including JAK, p38 MAPK, Wnt/β-catenin, JNK, ER stress and mitochondria-associated signal pathways. And we review five proteins interacting with IL-24, including Bip/GRP78, S1R, PKR, Beclin1 and soluble clusterin, which are relative to the tumor-specific effect of IL-24. It is speculated that ER stress, G-protein pathways and MAPK signal pathways may be the primary upstream effectors which activate the sequential downstream mediators resulting in apoptosis induced by IL-24 in tumor cells. Experimental results also show that IL-24 sensitizes cancer cells and indirectly promotes apoptosis rather than functions as a direct apoptosis inducer itself.

Key words: apoptosis; IL-24; signal pathway; tumor
CLC number: Q257

Received date: 2016-04-04
Foundation item: Supported by the National Program on Key Basic Research Project (973 Program) (2012CB518900), the National Natural Science Foundation of China (31160240, 31260621), the 12th Five Years Key Programs for Science and Technology Development of China (2012ZX10002006), the Hangzhou Normal University Supporting Project (PE13002004042), and the Natural Science Foundation of China of Jiangxi(20114BAB204016)
Biography: LIU Huilin, female, Master candidate, research direction: molecular mechanism of hepatocellular carcinoma and liver senescence, gene therapy in hepatocellular carcinoma. E-mail: liuhuilin1203@sina.com
† To whom correspondence should be addressed. E-mail: fanhandong@whu.edu.cn

0 Introduction

The melanoma differentiation-associated gene-7 (mda-7) was identified by performing subtraction hybridizations between different terminal differentiation melanoma cells in 1995 and renamed IL-24 based on the properties of a classical cytokine [1]. As a tumor suppressor gene with a potential ability in gene-based human cancer therapy, IL-24 is characterized by specifically inducing cancer apoptosis, a potent bystander effect, antiangiogenic properties and anti-tumor synergy with other treatments [2,3].

Both recombinant IL-24 and Ad.IL-24 induce cell growth suppression and apoptosis in extensive human cancer cells, without exerting any deleterious effects on their normal counterparts, including non-small cell lung carcinoma cells, colorectal cancer cells, ovarian cancer cells, prostate cancer cells, melanoma cells, breast cancer cells, glioma cells, oral cancer cells, hematopoietic malignancy cells, Hela cervical cancer cells, renal carcinoma cells, pancreatic cancer cells and lung cancer stem cells. Specially, the apoptosis induced by IL-24 is independent of classic tumor suppressor genes P53 and Rb [4-9].

Multiple signal pathways play a key role in IL-24-mediated apoptosis, including endoplasmic reticulum (ER) stress, reactive oxygen species (ROS) in the mitochondria, ceramide production, inhibition of β-catenin and PI3K, activation of p38 mitogen-activated protein kinase (MAPK), upregulation of pro-apoptotic proteins, downregulation of anti-apoptotic proteins, Fas-FasL signal, JAK, TLR3, Wnt/β-catenin [10].

For further researches about IL-24-mediate cancer therapy, we review the apoptotic signal pathways and

their regulatory mechanisms induced by IL-24 in cancer cells in this paper and summarize the basic characteristics of IL-24 signal networks in cancer cell-specific killing.

1 Structure and Receptors of Mda-7/ IL-24 Protein

Similar to other cytokine of IL-10 family, the gene encoding IL-24 is located on chromosome 1q32-33 in human genome with 7 exons and 6 introns. The cDNA of IL-24 includes 1 718 base pairs which encodes a 206 amino acid protein with a predicted molecular weight of 23.8 k [11]. The amino acid sequence from 1 to 49 is the putative signal peptide of IL-24 protein which is significant for the secretion of protein. Secreted IL-24 protein is different in molecular sizes because of the different N-glycosylation sites [12] at the putative amino acid residues 85, 99 and 126 [13] (Fig. 1). While studies reveal that the glycosylation of IL-24 is not mandatory for inducing cell death or bystander activities in different cancer cells [14].

Sequence analysis shows that there is an IL-10 signature region from amino acid residues 101 to 121, three protein kinase C (PKC) consensus phosphorylation sites located at amino acid residues 88, 133 and 161, and three casein kinase II (CKII) consensus phosphorylation sites on amino acid 101, 111 and 161 [13] (Fig. 1).

Using segmented analysis, a specific mutant of IL-24, consisting of amino acids 104 and 206, is necessary

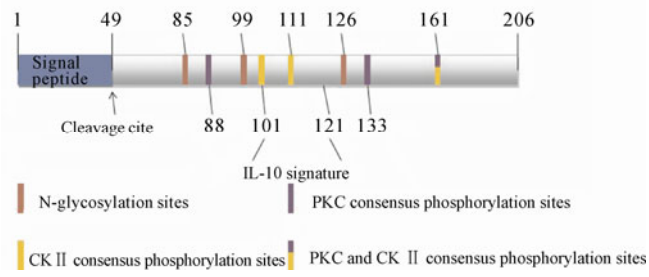

Fig. 1 Schematic representation of the primary structure of mda-7/IL-24 protein

There is a signal peptide from residues 1 to 49, which can be incised between residue 49 and residue 50 during secretion. Three putative glycosylation sites are located at amino acid residues 85, 99 and 126, three putative PKC consensus phosphorylation sites are located at amino acid residues 88, 138 and 161, and three putative casein kinase II (CKII) consensus phosphorylation sites are located at amino acid residues 101, 111 and 161. The primary sequence from amino acid residues 101 to 121 is the IL-10 signature sequence which is required for sequential series of responses

for the cancer-specific growth suppression and apoptosis-inducing properties of the full-length protein [15]. Two couples of receptors dimmers, IL-20R1/IL-20R2 and IL-22R1/IL-20R2, have been identified as IL-24 receptors which are responsible for activating the downstream signal cascades JAK/STAT [16] (Fig. 2). However, the researches of expression pattern of the receptors indicate that complete IL-24 receptors are seldom expressed in cancer cell lines [17], and IL-24 is independent of receptor expression and JAK/STAT signal in selective killing a vast variety of cancer cells, which is in stark contrast to its "normal" physiological behaviors [10].

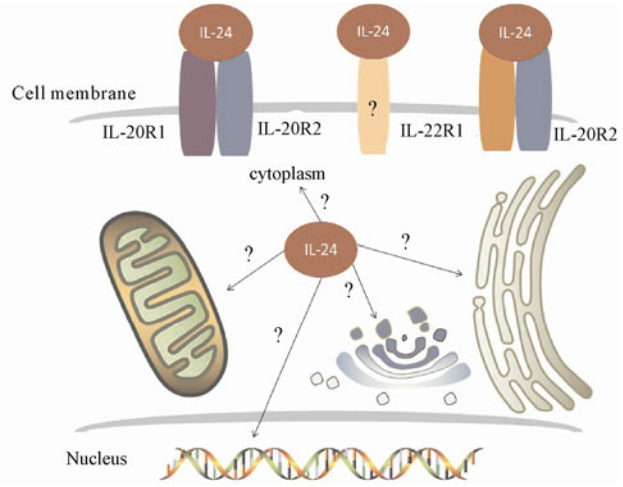

Fig. 2 Schematic of receptors or other putative targets of IL-24

The two couple of receptors, IL-20R1/IL-20R2 and IL-22R1/IL-20R2, are two homologous dimmers actually. IL-24 may have other targets, including possible new receptors

2 Cancer-Specific Apoptosis Induced by IL-24

Apoptosis, also known as programmed cell death, requires a sequential series of responses so that it can eliminate unwanted cells from body system. There are abundant evidences which demonstrate that IL-24 can selectively kill cancer cells and suppress tumor growth and migration without hurting normal cells [18]. Furthermore, IL-24 is found out to be nontoxic to normal stem cells, but toxic to cancer-initiating stem cells, thus providing further evidence for a possible application in cancer therapy [19].

2.1 Apoptosis Induced by Recombinant or Secreted IL-24

It have been demonstrated that the recombinant and secreted IL-24 can activate a series of intracellular downstream signaling by binding to IL-20R1/IL-20R2 and

IL-22R1/IL-20R2 [16]. There are also evidences that receptors are the key for IL-24 mediates-apoptosis signaling. Recombinant His-IL-24, combined with its receptor pairs, can induce prostate carcinoma cancer and pancreatic cancer cells apoptosis by the activation of p38 MAPK and subsequently induce the expression of SARI (suppressor of AP-1, regulated by IFN) (Fig. 3: A-1, A-1-2). Actually, SARI represents a downstream target of IL-24 in cancer cells and is functioned as a key determinant of cancer-specific killing [6].

In other studies, recombinant GST-IL-24 induces ceramide-dependent activation of Fas-FasL pathways which promotes multiple pro-apoptotic pathways, such as the phosphorylation of kinase RNA-like endoplasmic reticulum kinase (PERK), the activation of c-Jun N-terminal kinase1/2 (cJNK1/2) and p38 MAPK, the inactivation of ERK1/2 and autophagy to decrease survival in kidney cancer cells, A549 cells and glioma cells [20-23] (Fig. 3: E-1, 2, 3). Otherwise, the recombinant secreted IL-24 (commercial IL-24, GST-IL-24 expressed by bacteria, IL-24 secreted from transfected HEK cells, or transient overexpression of different IL-24 constructs) does not induce substantial apoptosis in several melanoma cells compared with that in the appropriate control treatments. But it should be noted that the three investigated cell lines including A375, MeWo and Wm35 could not express sufficient amounts of functional receptor pairs [10, 24].

Three same melanoma cell lines(A375, MeWo and Wm35) are used in another study, in which the secreted IL-24 triggered apoptosis of the cell types and the receptor pairs are detected on the membrane of the three cell lines [10, 24]. It is normal that the same cell line has different expression models for some proteins in different labs. And these results still provide support that the tumor specific killing activity of IL-24 is dependent on the receptors pairs. However, conflicting data have also been obtained. At lower concentrations, IL-24 activates STAT transcript 3 (STAT-3) and promotes cellular proliferation [25]. While, at higher concentrations (100-200 times increased), IL-24 activates STAT-1 and leads to cell growth inhibition [25].

Although the receptor pairs exist and STAT-3 can be activated in treatment with recombinant IL-24, the knock-down of STAT-3 in pancreatic tumor cells does not abrogate IL-24 protein- mediated cytotoxicity indicating that STAT-3 is not re- quired for IL-24-mediated antitumor activity [26] (Fig. 3: B, B-1, B-2). These results show that the initial IL-24- induced apoptosis of cancer cells is independent of JAK/ STAT signal pathways [10, 24]. Additionally, the secreted IL-24, extracellularly administered, fails to induce activation of PKR and has no cancer-specific apoptosis- inducing properties on H1299 lung tumor cells [23, 27]. But the investigators do not check the receptor pairs in the H1299 cell membrane.

In fact, many cancer cells and human tissues lack sufficient amounts of functional receptor pairs and therefore do not react to recombinant and secreted IL-24 treatments [10, 17]. This throws into doubt whether IL-24 can exert so-called bystander effects on cancer cells without receptor pairs if the presence of receptors is necessary [23, 26]. Immune-modulatory function of IL-24 may play another important role in promoting the killing signals. IL-24 is preferably secreted by monocytes, T cells and B cells, and increasingly expressed in the position of immune- opathology.

The secreted IL-24 protein can activate human peripheral blood mononuclear cell (PBMC, via STAT3 phosphorylation) and induce secretion of IFN-γ, IL-6, and TNF-α, and along with lower levels of IL-1, IL-12, and granulocyte-macrophage colony stimulating factor (GM-CSF) from human PBMCs favoring a Th1-type immune response [28]. It might be these pro-in flammatory cytokines as well as activated immune cells that provide the killing signals of cancer and function as bystander effecters [29]. These studies provide further support for a mode of killing by IL-24 that involved in intracellular actions and the secreting IL-24 functioned as a synergy effect especially when the targeted cell has IL-24 receptor pairs.

2.2 Functions of Expression Vectors Delivering IL-24

Ad.IL-24 induces growth suppression and cancer-specific apoptosis in a panel of different cancer cells without doing harm to their normal counterparts [4-9]. These studies, combined with experiments results that adenovirus delivering IL-24 lacking a signal peptide also induces cancer-specific apoptosis in A549 cells, provide further support for the combination of IL-24 with adenoviral overexpression vector that represents a promising treatment choice for various solid cancers [30]. Not requiring for viral replication, IL-24-mediated enhancement of the influenza A virus also induces cell death, which is mediated by TLR3 and the formation of an atypical TLR3-associated death-inducing signaling complex(DISC), followed by the activation of caspase-8 and subsequent caspase-3(Fig. 3: C).

cFLIP, one of the ingredients of DISC, plays a

major role in regulation of cell death induced by TLRs and is dissociated from the atypical death complex DISC in the presence of IL-24, which makes TLR-mediated apoptosis to be possibly not restricted to the stimulation of TLR3 [31]. As adenoviruses are known to stimulate TLR2 and TLR9, influenza A virus, similar to adenoviruses, probably provides a starting stimulus, while IL-24 promotes an additional stimulus to induce apoptosis by interfering the atypical death complex.

2.3 JAK/STAT Pathway

Some researches verified that Ad.IL-24 can activate JAK and its following response STAT3 in diverse human cancer cells, including melanoma, breast, prostate, fibrosarcoma cancer cells and Hela cervical cancer cells [7, 10, 32] (Fig. 3: B). The activated JAK/ STAT3 upregulates the expression of IFN regulatory factor 2 (IRF-2) and downregulates the expression of both IFN regulatory factor 1 (IRF-1) and inducible nitric oxide synthase (iNOS) in melanoma [33] (Fig. 3: B, B-1). Both IRF1 and IRF2 are nuclear transcription factors in response to IFN-γ, but the functions of the two factors are contradictory: IRF-1 induces the expression of iNOS, while IRF-2 inhibits the expression of iNOS [34]. As a result, the activated JAK/STAT3 attenuates the expression of iNOS, thus inhibiting tumor growth and metastasis. Meanwhile, the activated JAK/STAT3 also upregulates growth arrest-specific gene-3 (gas3) to inhibit the interaction between β1 integrin and fibronectin, which inhibits the attachment and proliferation of breast cancer cells [35] (Fig. 3: B, B-2).

Although Ad.IL-24 treatment can activate JAK/ STAT3 and induce tumor cell apoptosis, the apoptosis is not always dependent on the activation of JAK/STAT3. Treatment with distinctive tyrosine kinase inhibitors (Genistein and AG18) or JAK-selective inhibitor (AG490) does not prevent apoptosis induced by Ad.IL-24 in diverse cell lines (C8161, DU145, and HO-1) [32]. The recombinant His-IL-24 can induce the same prostate carcinoma by the activation of p38 MAPK but it is independent of JAK/STAT [6] (Fig. 3: A-1, A-1-2). In addition, recombinant or secreted IL-24 does not induce apoptosis of some melanoma cells, and the apoptosis induced by Ad.IL-24 is independent of JAK/STAT3 signal pathways and receptor engagement [10, 24].

2.4 MAPK Pathway

MAPK signal transduction pathways are evolutionarily conserved among eukaryotes and consist of 5 main subgroups of MAPKs: extracellular signal- regulated kinase-1/2 (ERK1/2), c-Jun NH_2-terminal kinase (JNK / SAPK), p38 MAPK (p38α, p38β, p38γ and p38δ), ERK3/4 and ERK5. JNKs, activated by MKK7/4, are encoded by three genes, JNK1, JNK2 and JNK3. p38 MAPK is selectively activated by MAPK kinases 3/6 (MKK3/6). Both JNKs and p38 MAPK are important mediators of apoptosis, while ERK1/2 are key transducers of proliferation, differentiation and survival signals [36]. p38 MAPK is associated with Ad. IL-24-induced cancer cell-specific killing in melanoma cells, glioblastoma multiforme, prostate cancer, breast cancer, pancreatic cancer and ovarian cancer cells, and the activated p38 MAPK promotes the transcription of a family of growth arrest and DNA damage (GADD) including GADD153, GADD34, GADD45α and GADD45γ, and GADD153 but not in corresponding counterparts [37-39] (Fig. 3: D, A-1).

GADD34 is involved in translation initiation, DNA recombination or repair, mRNA transportation, and transcriptional regulation by interacting with a diverse array of proteins within the cell. GADD45 functions in nucleotide excision are repaired by associating with p21 WAF1/CIP1/MDA-6 and PCNA (proliferating cell nuclear antigen) and mediate activation of p38 and JNK MAPK by interacting with MAPK kinase kinases (MAPKKKs). GADD153 primarily interferes with C/EBP-mediated transcription. The downregulating Bcl-2 may be mediated by GADD153 with down-regulating the Bcl-2 promoter [40]. Apart from GADD and Bcl-2, p38 MAPK also acts on the downstream target heat shock protein (HSP27), which initiates apoptosis [37]. p38 MAPK also stabilizes the mRNA of IL-24 and upregulates the expression of IL-24 as a regulator of IL-24 expression by stabilizing the 3'UTR of IL-24 mRNA [40]. But p38 MAPK should have no effect on the stabilization of mRNA which is transcribed from the DNA of Ad.IL-24, because the transcription variant has no 3'UTR. The activation and upregulation of protein kinase R (PKR) appear to be another crucial upstream signal cascade in Ad.IL-24 inducing apoptosis in some cancer cells such as lung cancer cells, ovarian cancer, leukemia and melanoma[11, 40, 41], which correspondingly results in phosphorylation of its down-stream targets eIF-2α, Tyk2, STAT1, STAT3, JNK and p38 MAPK [13, 38, 42]. It has been demonstrated that IL-24 can physically interact with PKR in lung cancer cells and PKR as well as IL-24 (on threonine and serine residues) is phosphorylated by treatment with Ad.IL-24 [43].

It should be noted that secreted IL-24 cannot activate the PKR pathway in human melanoma cells,

A549 and H1299 NSCLC cell lines, and its bystander effects are independent of activation of PKR [23]. The death-inducer Fas ligand and its cognate receptor Fas (Fas-FasL) can be induced by Ad.IL-24 via JNK cascade in ovarian cancer cells, in which activated JNK excites transcription factor c-Jun and ATF2. Then the two factors would shift their location to nucleus to initiate the transcription of Fas and FasL [38] (Fig. 3: H-1, H-2). The secreted FasL is associated with Fas and Fas-FasL complex promoted to recruit DISC, which results in cell death via activating caspase-8 [38] (Fig. 3: H-3). However, whether cFLIP is dissociated from the DISC has not been checked in the study. In addition, Ad.IL-24 induces a remarkable increase of various ceramides (C16, C24, C24:I) in prostate cancer cells but not in normal cells by increasing the expression and activity of acid sphingomyelinase (AMSase) and translocation of ASMase from the endosomal/lysosomal compartments to the plasma membrane [44-46] (Fig. 3: E).

The increasing ceramide may form lipid rafts in the plasma membrane to promote the formation of Fas and TRAIL receptor clustering and thereby play a prominent role in Ad.IL-24 enhanced apoptotic signal [47, 48] (Fig. 3: E-1). Ad.IL-24 upregulates pro-apoptotic protein Bim and protein phosphatase 2A (PP2A) via upregulating ceramide production. As a result, Bim leads to mitochondria dysfunction and PP2A inhibits the activetion of extracellular regulated kinase (ERK) and v-AKT murine thymoma viral oncogene homolog (AKT) [44-46] (Fig. 3: E, E-2). The rising ceramide also increases ROS which correspondingly aggravate the oxidative stress and ER stress [49] (Fig. 3: E-3). Other reviews disclose that ceramide treatment in a prostate and colorectal cancer cell line induces apoptosis only when Bax is overexpressed, suggesting that ceramide might not be the final apoptosis executors whose functions are dependent on other apoptosis mediators [47].

2.5 ER Stress Pathways and Mitochondria-Mediated Apoptosis with Treatment of Ad.IL-24

The endoplasmic reticulum (ER) is an essential organelle in eukaryotic cells for processing and modifying protein, storing calcium and conducting the entrance of secreted protein. With the accumulation of misfolding/unfolding proteins, the steady state is disturbed and ER stress is induced, which may lead cancer cells to apoptosis [50]. The unfolding protein response (UPR) consists of three main signal sensors: double-stranded RNA-activated protein kinase-like ER kinase (PERK), inositol-requiring kinase 1α (IRE1α) and activating transcription factor 6(ATF6) [51, 52] (Fig. 3: F, G, H).

Under non-stressed conditions, BiP/GRP78 is bound to the luminal domains of the three sensors to prevent their dimerization. However, the unfolding/misfolding protein has stronger interaction with BiP/GRP78 than the three UPR sensors and makes BiP/GRP78 to be disassociated from them. Subsequently, the three sensors form dimers and are activated. Activated PERK phosphorylates eukaryotic translation initiation factor 2 on the α subunit(eIF2α) and attenuates global translation [52] (Fig. 3: F, F-1).

EIF2α phosphorylation also can activate transcription factor 4(ATF4) to translocate to nucleus for transcription of many apoptosis-associated gene, such as GADD family and CAAT/enhancer-binding protein (C/EBP) homologous protein (CHOP) [53, 54] (Fig. 3: F-1-1). Spliced ATF6 are also transported into nucleus then binds to the ATF/cAMP response element (CRE) and the ER stress-response element (ERSE-1) to activate target genes, such as CHOP [55] (Fig. 3: H).

Activated IRE1α cleaves XBP1 mRNA by removing a 26-nucleotide intron and this splicing reaction creates a translational frame shift to translate the protein XBP1 functioned as a transcription factor to activate CHOP [52] (Fig. 3: G-1) as well as a kinase to activate IκB kinase(IKK) [56] (Fig. 3: G-2), apoptosis signal-regulating kinase 1 (ASK1) and JNK [50]. Those activations upregulate the expression of pro-apoptotic protein such as Bim and downregulate the expression of anti-apoptotic protein such as Bcl-2 [50] (Fig. 3: G-3).

Accumulation of evidences suggests that IL-24 can activate ER stress efficiently, and ER stress-mediate signal pathway is vital in Ad.IL-24-induced apoptosis[15, 57-59]. The experimental data in human prostate carcinoma, cervical cancer and breast cancer cell lines demonstrate that IL-24 interacts with BiP/GRP78 through their C and F helices and culminates in cancer selective apoptosis [15] (Fig. 3: I). In addition, sigma 1 receptor (S1R), a endoplasmic reticulum protein, was recently identified to have physical interaction with IL-24. It is a critical initial mediator involved in Ad.IL-24-induced ER stress, ROS production, caspases-3 activation, and calcium mobilization in human lung cancer cells [57] (Fig. 3: J). Furthermore, S1R have been identified to bind to BiP and regulated ER stress, calcium mobilization, ROS and ceramide production [60], which provides an explanation for how IL-24 induces cell death without the need of cell-surface receptors and why internalized IL-24 has the function of

inducing apoptosis. Activated PERK by Ad.IL-24 results in ROS production and the increase of intracellular ceramide levels.

The increase of intracellular ceramide further facilitates calcium depend-generation of ROS production. Both of them lead to the promotion of mitochondrial dysfunction and cell death, which can be antagonized by antioxidants and synergized by agents that induce ROS production [61, 62]. Inhibition of ERK 1/2 and activation of both JNK and nuclear factor kappa B (NF-κB) have been demonstrated by ER stress induced by Ad.IL-24 [46, 56]. Activated PERK primarily focuses on inhibition of ERK 1/2 and activation of JNK, while activates IRE1α contributes to activate NF-κB by phosphorylating IKK (Fig. 3: F-2, G-2). The activation of JNK and NF-κB, combined with phosphorylation of eIF2*α*, plays a prominent role in upregulation of pro-apoptotic proteins and downregulation of anti-apoptotic proteins, including Bax, Bcl-2, TRAF2, FasL, Bcl-xl, TNF-α, GADDs, Mcl-1 and c-Flip [3, 52, 63-66].

The leakage of Ca^{2+} from ER to mitochondria and the release of cytochrome C from mitochondria to cytoplasm are two significant events in mitochondria-mediated apoptosis. The upregulated pro-apoptotic proteins and downregulated anti-apoptotic proteins mentioned above ultimately act on mitochondria. For example, pro-apoptotic protein Bax and Bak aggregate on mitochondria membrane to open the permeability transition pore which provides the entrance of Ca^{2+} and the exit of cytochrome C [67-69] (Fig.3: K). Down- regulation of Bax or ectopic expression of Bcl-2 inhibits the release of Ca^{2+} and ROS-mediated cell death in tumor cells but not in normal cells when cells are treated with Ad.IL-24 [49] (Fig.3: K).

ROS can also upregulate pro-apoptotic protein Bak and activate caspase-2/4 via phosphorylating JNK [3, 46] (Fig. 3: L). It is believed that cross talk between ER and mitochondria co-operatively promotes the cell death [56]. These results are unveiled with a new observation that ER stress induced by Ad.IL-24 can be the common upstream events and their subsequential downstream targets are p38 MAPK, JNK, NF-κB, ERK1/2, calcium mobilization, ROS, and ceramide production. These proteins are up/ downregulated by the downstream targets function as apoptosis executors.

2.6 Autophagy

It is evident that autophagy can be detected in the process of cell apoptosis induced by both Ad.IL-24 and recombinant IL-24 [62, 70]. Moreover, Ad.IL-24 can switch prostate cancer cells from autophagy to apoptosis by inducing the expression of autophagy-related genes Beclin1 and calpain-mediated cleavage of the Atg5 protein [70]. Intracellular IL-24 can physically interact with Beclin1, and this interaction might inhibit Beclin1 function and culminate in apoptosis [70].

As described above, ER stress induced by Ad.IL-24 can activate p38 MAPK and JNK as well as inhibit ERK1/2. We speculate that it might be the activated MAPK and the inhibited ERK1/2 that promote autophagy signal cascades, and the interaction between IL-24 and Beclin1 enhances the strength of signal and makes it to be irreversible. And then autophagy is just one of the downstream signals of ER stress. However, one study shows that IL-24 suppresses the chemokine CXCL12/ CXCR4 axis, resulting in the inactivation of CXCL12/ CXCR4-Akt-mTOR and the corresponding activated autophagy [62].

Previous reports also demonstrate that CXCL12/ CXCR4 induces G protein-coupled signal pathways, such as phosphoinositide 3-kinase (PI3K)/AKT, Rac1, Rho, MAPK and activator protein-1(AP-1), which subsequently mediates cellular responses [71]. And pertussis toxin treatment suppresses IL-24-induced migration, implicating G-protein coupled receptors are relevant in this process [72]. These results suggest that IL-24 may contribute to the known anti-cancer effects by activating G-protein signal pathways, but more evidences are required to indicate signal pathways cross talks induced by Ad.IL-24 among CXCL12/CXCR4 pathway, G protein-coupled signal pathways and ER stress.

2.7 Wnt/*β*-Catenin Pathway

There are also some literatures indicate that Ad.IL-24 can downregulate breast cancer stem cells self-renewal capacity by suppressing Wnt/*β*-catenin pathway [19]. GSK3β is activated by Ad.IL-24 phosphorylate *β*-catenin at Ser33/Ser37/Thr41, thus, promote ubiquitin-proteasome degradation of *β*-catenin and downregulate Wnt/*β*-catenin pathway (Fig. 3: M). Therefore, IL-24 suppresses breast cancer-initiating/stem cells' self-renewal ability by weakening Wnt/*β*-catenin pathway which is also detected in malignant melanoma cells [19, 73]. Moreover, transduction of Ad. IL-24 increases the expression of proteins that regulates Wnt/PI3K pathway, including tumor suppressor proteins APC, GSK-3*β*, PTEN and the Frizzled receptor. While β-catenin, the target protein of Wnt, is downregulated [26]. Negative regulation of *β*- catenin and PI3K pathways is another process involved in apoptosis induced by IL-24 in human breast, lung and

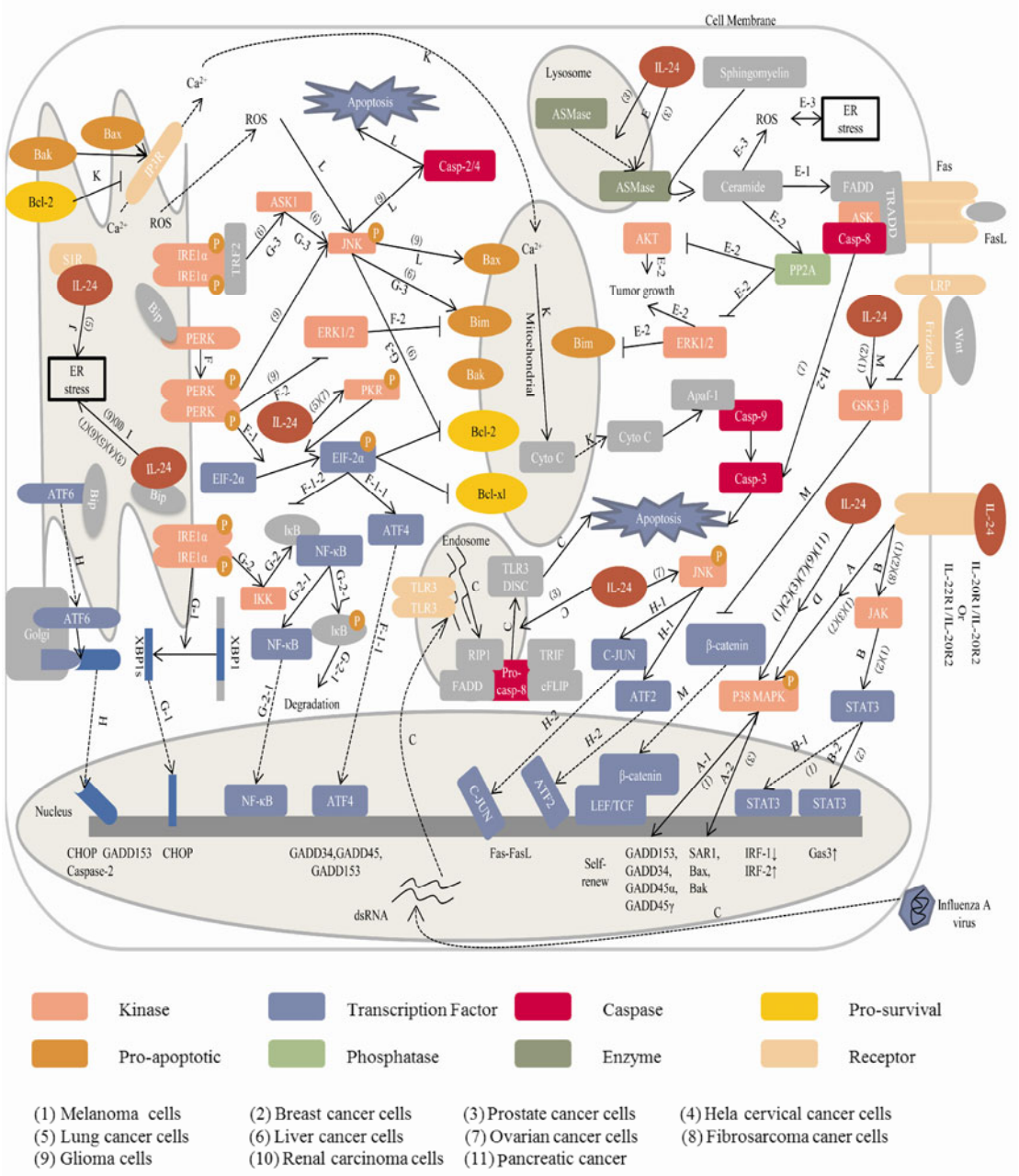

Fig. 3 Schematic representation of the general signal pathway in mda-7/IL-24-induced apoptosis in cancer cells

Serial numbers from (1) to (10) represent various human cancer cells respectively, which show the corresponding pathways reported in particular cells; A to M represent the signal pathways, the dotted lines represent translocation

pancreatic cancer cells [26]. Experimental data also show that JNK is the downstream of Wnt/PI3K because of the upregulation of JNK1 and JNK2 in pancreatic tumor cells transfected with Ad.IL-24 [26].

3 Summary

Ad.IL-24 and IL-24 effectively induce apoptosis of extensive tumor cells in relation to multiple signal pathways, while the precise regulating mechanisms have not been understood clearly. Even so, some clues can also be summarized from these obtained data: ER stress and MAPK activation induced by Ad.IL-24 should be the primary upstream signals of apoptosis in cancer cells [15, 37]. As for cancer cell toxicity induced by Ad.IL-24 and IL-24, the expression of multiple pro-apoptotic and anti-apoptotic proteins enhance ER stress, mitochondrial dysfunction, autophagy and ROS level, and finally results in apoptosis of cancer cells. In addition, G-protein and PKR signals are also involved in anti-cancer effects induced by Ad.IL-24, however, researchers have not focused on the cross talks among ER stress, MAPK

pathway, PKR and G-protein cascade so far [15, 37, 43, 72]. Further studies are needed to identify the upstream molecule mechanism for delineating the relevant signal transduction pathways networks and regulation mechanisms involved in Ad.IL-24 and IL-24-induced apoptosis. IL-24 is shown to be a cell-type dependent apoptosis inducer which is different from other pro-apoptosis proteins or chemical molecules. The most confused characteristic of IL-24 is related to dependency/independency on JAK/STAT signal pathway and PKR activation. The apoptosis induced by Ad.IL-24 and IL-24 proteins are not always dependent on/relative to the activation of JAK/STAT3 [7, 32]. Next, we will discuss the possible reasons of cancer cells-specific apoptosis induced by IL-24 from three aspects.

1) Inherent biochemical differences between normal and cancer cells lead to different responses to IL-24

Although the mechanisms of apoptosis induced by Ad.IL-24 are gradually understood, the question why IL-24 has the abilities to selectively induce apoptosis in human cancer-derived cell lines without doing harm to normal cells remains unresolved. Inherent biochemical differences between normal and cancer cells could be a possible reason for the disparate effects, such as ceramide and pro-apoptosis/anti-apoptosis proteins. S1R which interacts with IL-24 and has higher expression in cancer cells has been identified to be a critically initial mediator in Ad.IL-24-induced alternative apoptosis in prostate cancer cell lines [57], while the same mechanism is not proved in other tumor cells. Ad.IL-24 can also indirectly mediate the expression of proteins by regulating alternative splice of mRNA in cancer cells. Clusterin, another interactional protein of IL-24, has been classified into two protein isoforms, sCLU and nCLU, because of alternative splice of mRNA. sCLU is a pro-survival secreted protein mainly localized in the cytoplasm, but nCLU is a pro-apoptotic protein mainly localized in the nucleus in human cells [74-78]. The experimental data shows that Ad.IL-24 decreases expression of sCLU but increases expression of nCLU by affecting alternative splice of mRNA of CLU in prostate cancer cell. By these regulations of mRNA, Ad.IL-24 promotes G2/M phase arrest followed by apoptosis [79]

2) Cancer cells have insufficient environmental adaptive ability

Another possible reason of cancer cell-specific apoptosis induced by IL-24 is the differences in the ability to maintain cell homeostasis and recovery from the dyshomeostasis between normal and cancer cells. As a result of adapting to the unfavorable microenvironments with low pH, low oxygen, or other nutrient levels, cancer cells evolve autophagy, UPR, ROS and anaerobic respiration [80]. This adaptive capacity also makes tumor cells easier to be disturbed and more difficult to recover from dyshomeostasis. Autophagy could be induced by Ad.IL-24 in prostate cancer cell [79]. And IL-24 induces apoptotic effect through ER stress mechanisms exclusively in cancer cells [81]. ER stress activates both pro-survival and pro-apoptotic signals, however, a very strong or prolonged ER stress can overwhelm the pro-survival mechanism and incline to apoptotic pathways. Compared with normal cells, cancer cells have higher ER stress levels which cause them more susceptible to ER stress induced by IL-24 [42].

3) IL-24 increases sensibility of cancer cells

ROS, a key mediator of IL-24 effects, have differential effects on cancer cells versus normal cells. Multiple studies demonstrate that the basal ROS level in cancer cells is higher than that in normal cells [81]. The virus vectors also play a vital function in cancer-specific apoptosis-inducing properties of IL-24. As adeno-viruses are known to primarily stimulate TLR2 and TLR9, influenza A virus, similar to adenoviruses, probably provides a starting stimulus, while IL-24 promotes an additional stimulus to induce apoptosis by interfering the atypical death complex [31]. A cascade effect can lead to activation of multiple apoptotic signals, thus enhance this effect up to a point of irreversible commitment and cell death. IL-24 decreases the presence of cFLIP in the TLR3-associated complex containing TRIF, RIP1, FADD, cFLIP and pro-caspase-8 and converts it into an atypical TLR3-associated death-inducing signaling complex with treatment with an influenza A virus vector expressing IL-24 [31]. Therefore, rather than acting as an apoptosis inducer itself, IL-24 sensitizes cancer cells to both internal and external stimuli. IL-24 combined with tumor-suppressing drugs like tarceva and dacarbazine can enhance the cancer cell death compared with respective tumor-suppressing drugs [3, 82, 83].

However, a detailed analysis of the molecular mechanism underlying this effect has not been performed. Understanding these significant properties of IL-24 will facilitate the development of rational combinatorial approaches with potential to enhance therapeutic activity. In addition, it is discovered that IL-24 protein generation expressed with adenoviral vectors is low or absent in some normal cells compared to cancer cells [11, 84]. Highly activated MEK1/2/ERK1/2 in normal cell lines results in

"translational block", which has been observed in mutant K-ras pancreatic cancer cells under treatment with Ad.IL-24 [85], but no investigation focuses its attention on the kind of translational block in other cells.

References

[1] Jiang H, Lin J J, Su Z Z, *et al*. Subtraction hybridization identifies a novel melanoma differentiation associated gene, mda-7, modulated during human melanoma differentiation, growth and progression [J]. *Oncogene*, 1995, **11**(12): 2477-2486.

[2] Lee K M, Kang H A, Park M, *et al*. Interleukin-24 attenuates beta-glycerophosphate-induced calcification of vascular smooth muscle cells by inhibiting apoptosis, the expression of calcification and osteoblastic markers, and the Wnt/beta-catenin pathway [J]. *Biochem Biophys Res Commun*, 2012, **428**(1): 50-55.

[3] Dent P, Yacoub A, Hamed H A, *et al*. The development of mda-7/IL-24 as a cancer therapeutic [J]. *Pharmacol Ther*, 2010, **128**(2): 375-384.

[4] Eulitt P J, Park M A, Hossein H, *et al*. Enhancing mda-7/IL-24 therapy in renal carcinoma cells by inhibiting multiple protective signaling pathways using sorafenib and by ad.5/3 gene delivery [J]. *Cancer Biology & Therapy*, 2014, **10**(12): 1290-1305.

[5] Kim J S, Yu S K, Lee M H, *et al*. Microrna-205 directly regulates the tumor suppressor, interleukin-24, in human KB oral cancer cells [J]. *Mol Cells*, 2013, **35**(1): 17-24.

[6] Dash R, Bhoopathi P, Das S K, *et al*. Novel mechanism of mda-7/IL-24 cancer-specific apoptosis through sari induction [J]. *Cancer Res*, 2014, **74**(2): 563-574.

[7] Sarkar D, Dent P, Curiel D T, *et al*. Acquired and innate resistance to the cancer-specific apoptosis-inducing cytokine, mda-7/IL-24: Not insurmountable therapeutic problems [J]. *Cancer Biol Ther*, 2008, **7**(1): 109-112.

[8] Hingtgen S, Kasmieh R, Elbayly E, *et al*. A first-generation multi-functional cytokine for simultaneous optical tracking and tumor therapy [J]. *PLoS One*, 2012, **7**(7): e40234.

[9] Sauane M, Su Z Z, Dash R, *et al*. Ceramide plays a prominent role in mda-7/IL-24-induced cancer-specific apoptosis [J]. *J Cell Physiol*, 2010, **222**(3): 546-555.

[10] Kreis S, Philippidou D, Margue C, *et al*. Recombinant interleukin-24 lacks apoptosis-inducing properties in melanoma cells [J]. *PLoS One*, 2007, **2**(12): e1300.

[11] Huang E Y, Madireddi M T, Gopalkrishnan R V, *et al*. Genomic structure, chromosomal localization and expression profile of a novel melanoma differentiation associated (mda-7) gene with cancer specific growth suppressing and apoptosis inducing properties [J]. *Oncogene*, 2001, **20**(48): 7051-7063.

[12] Wang M, Tan Z, Zhang R, *et al*. Interleukin 24 (mda-7/mob-5) signals through two heterodimeric receptors, IL-22r1/IL-20r2 and IL-20r1/IL-20r2 [J]. *J Biol Chem*, 2002, **277**(9): 7341-7347.

[13] Sauane M, Gopalkrishnan R V, Sarkar D, *et al*. Mda-7/IL-24: Novel cancer growth suppressing and apoptosis inducing cytokine [J]. *Cytokine Growth Factor Rev*, 2003, **14**(1): 35-51.

[14] Sauane M, Gupta P, Lebedeva I V, *et al*. N-glycosylation of mda-7/IL-24 is dispensable for tumor cell-specific apoptosis and "bystander" antitumor activity [J]. *Cancer Res*, 2006, **66**(24): 11869-11877.

[15] Gupta P, Walter M R, Su Z Z, *et al*. BIP/GRP78 is an intracellular target for mda-7/IL-24 induction of cancer-specific apoptosis [J]. *Cancer Res*, 2006, **66**(16): 8182-8191.

[16] Dumoutier L, Leemans C, Lejeune D, *et al*. Cutting edge: STAT activation by IL-19, IL-20 and mda-7 through IL-20 receptor complexes of two types [J]. *J Immunol*, 2001, **167**(7): 3545-3549.

[17] Zhu H, Yang Z B. Expression pattern of mda-7/IL-24 receptors in liver cancer cell lines [J]. *Hepatobiliary Pancreat Dis Int*, 2009, **8**(4): 402-406.

[18] Yang Y J, Chen D Z, Li L X, *et al*. Targeted IL-24 gene therapy inhibits cancer recurrence after liver tumor resection by inducing tumor cell apoptosis in nude mice [J]. *Hepatobiliary Pancreat Dis Int*, 2009, **8**(2): 174-178.

[19] Bhutia S K, Das S K, Azab B, *et al*. Targeting breast cancer-initiating/stem cells with melanoma differentiation-associated gene-7/interleukin-24 [J]. *Int J Cancer*, 2013, **133**(11): 2726-2736.

[20] Park M A, Walker T, Martin A P, *et al*. Mda-7/IL-24-induced cell killing in malignant renal carcinoma cells occurs by a ceramide/CD95/PERK-dependent mechanism [J]. *Mol Cancer Ther*, 2009, **8**(5): 1280-1291.

[21] Yacoub A, Park M A, Gupta P, *et al*. Caspase-, cathepsin-, and PERK-dependent regulation of mda-7/IL-24-induced cell killing in primary human glioma cells [J]. *Mol Cancer Ther*, 2008, **7**(2): 297-313.

[22] Sauane M, Gopalkrishnan R V, Choo H T, *et al*. Mechanistic aspects of mda-7/IL-24 cancer cell selectivity analysed via a bacterial fusion protein [J]. *Oncogene*, 2004, **23**(46): 7679-7690.

[23] Chada S, Mhashilkar A M, Ramesh R, *et al*. Bystander activity of ad-mda7: Human mda-7 protein kills melanoma cells via an IL-20 receptor-dependent but stat3-independent mechanism [J]. *Mol Ther*, 2004, **10**(6): 1085-1095.

[24] Parrish-Novak J, Xu W, Brender T, *et al*. Interleukins 19, 20 and 24 signal through two distinct receptor complexes. Differences in receptor-ligand interactions mediate unique biological functions [J]. *J Biol Chem*, 2002, **277**(49): 47517-47523.

[25] Inoue S, Shanker M, Miyahara R, *et al*. Mda-7/IL-24-based cancer gene therapy: Translation from the laboratory to the clinic [J]. *Curr Gene Ther*, 2006, **6**(1): 73-91.

[26] Chada S, Bocangel D, Ramesh R, *et al*. Mda-7/IL-24 kills pancreatic cancer cells by inhibition of the Wnt/PI3K signaling pathways: Identification of IL-20 receptor-mediated bystander activity against pancreatic cancer [J]. *Mol Ther*, 2005, **11**(5): 724-733.

[27] Sieger K A, Mhashilkar A M, Stewart A, *et al*. The tumor suppressor activity of mda-7/IL-24 is mediated by intra-cellular protein expression in nsclc cells [J]. *Mol Ther*, 2004, **9**(3): 355-367.

[28] Sahoo A, Lee C G, Jash A, *et al*. Stat 6 and c-jun mediate Th2 cell-specific IL-24 gene expression [J]. *J Immunol*, 2011, **186**(7): 4098-4109.

[29] Shefler I, Pasmanik-Chor M, Kidron D, *et al*. T cell-derived microvesicles induce mast cell production of IL-24: Relevance to inflammatory skin diseases [J]. *J Allergy Clin Immunol*, 2014, **133**(1): 1-3.

[30] Sarkar D, Lebedeva I V, Gupta P, *et al*. Melanoma differentiation associated gene-7 (mda-7)/ IL-24: A "magic bullet" for cancer therapy? [J]. *Expert Opin Biol Ther*, 2007, **7**(5): 577-586.

[31] Weiss R, Sachet M, Zinngrebe J, *et al*. IL-24 sensitizes tumor cells to TLR3-mediated apoptosis [J]. *Cell Death Differ*, 2013, **20**(6): 823-833.

[32] Sauane M, Gopalkrishnan R V, Lebedeva I, *et al*. Mda-7/IL-24 induces apoptosis of diverse cancer cell lines through JAK/STAT-independent pathways [J]. *J Cell Physiol*, 2003, **196**(2): 334-345.

[33] Ekmekcioglu S, Ellerhorst J A, Mumm J B, *et al*. Negative association of melanoma differentiation-associated gene (mda-7) and inducible nitric oxide synthase (INOS) in human melanoma: Mda-7 regulates INOS expression in melanoma cells [J]. *Mol Cancer Ther*, 2003, **2**(1): 9-17.

[34] Mamane Y, Heylbroeck C, Genin P, *et al*. Interferon regulatory factors: The next generation [J]. *Gene*, 1999, **237**(1): 1-14.

[35] Li Y J, Liu G, Li Y, *et al*. Mda-7/ IL-24 expression inhibits breast cancer through upregulation of growth arrest-specific gene 3 (gas3) and disruption of 1 integrin function [J]. *Molecular Cancer Research*, 2013, **11**(6): 593-603.

[36] Lei Y Y, Wang W J, Mei J H, *et al*. Mitogen-activated protein kinase signal transduction in solid tumors [J]. *Asian Pac J Cancer Prev*, 2014, **15**(20): 8539-8548.

[37] Sarkar D, Su Z Z, Lebedeva I V, *et al*. Mda-7 (IL-24) mediates selective apoptosis in human melanoma cells by inducing the coordinated overexpression of the GADD family of genes by means of p38 MAPK [J]. *Proc Natl Acad Sci USA*, 2002, **99**(15): 10054-10059.

[38] Gopalan B, Litvak A, Sharma S, *et al*. Activation of the Fas-FasL signaling pathway by mda-7/IL-24 kills human ovarian cancer cells [J]. *Cancer Res*, 2005, **65**(8): 3017-3024.

[39] Sarkar D, Su Z Z, Lebedeva I V, *et al*. Mda-7 (IL-24): Signaling and functional roles [J]. *Biotechniques*, 2002, **Suppl**: 30-39.

[40] Otkjaer K, Holtmann H, Kragstrup T W, *et al*. The p38 MAPK regulates IL-24 expression by stabilization of the 3'UTR of IL-24 mRNA [J]. *PLoS One*, 2010, **5**(1): e8671.

[41] Qian W, Liu J, Tong Y, *et al*. Enhanced antitumor activity by a selective conditionally replicating adenovirus combining with mda-7/interleukin-24 for B-lymphoblastic leukemia via induction of apoptosis [J]. *Leukemia*, 2008, **22**(2): 361-369.

[42] Gupta P, Su Z Z, Lebedeva I V, *et al*. Mda-7/IL-24: Multifunctional cancer-specific apoptosis-inducing cytokine [J]. *Pharmacol Ther*, 2006, **111**(3): 596-628.

[43] Pataer A, Vorburger S A, Chada S, *et al*. Melanoma differentiation-associated gene-7 protein physically associates with the double-stranded RNA-activated protein kinase PKR [J]. *Mol Ther*, 2005, **11**(5): 717-723.

[44] Yacoub A, Gupta P, Park M A, *et al*. Regulation of GST-mda-7 toxicity in human glioblastoma cells by ERBB1, ERK1/2, PI3K, and JNK1-3 pathway signaling [J]. *Mol Cancer Ther*, 2008, **7**(2): 314-329.

[45] Sauane M, Su Z Z, Dash R, *et al*. Ceramide plays a prominent role in mda-7/IL-24-induced cancer-specific apoptosis [J]. *Journal of Cellular Physiology*, 2009, **222**(3): 546-555.

[46] Yacoub A, Park M A, Gupta P, *et al*. Caspase-, cathepsin-, and PERK-dependent regulation of mda-7/IL-24-induced cell killing in primary human glioma cells [J]. *Molecular Cancer Therapeutics*, 2008, **7**(2): 297-313.

[47] Taha T A, Mullen T D, Obeid L M. A house divided: Ceramide, sphingosine, and sphingosine-1-phosphate in programmed cell death [J]. *Biochim Biophys Acta*, 2006, **1758**(12): 2027-2036.

[48] Park M A, Hamed H A, Mitchell C, *et al*. A serotype 5/3 adenovirus expressing mda-7/IL-24 infects renal carcinoma cells and promotes toxicity of agents that increase ROS and ceramide levels [J]. *Mol Pharmacol*, 2011, **79**(3): 368-380.

[49] Lebedeva I V, Su Z Z, Sarkar D, *et al*. Melanoma differentiation associated gene-7, mda-7/interleukin-24,

induces apoptosis in prostate cancer cells by promoting mitochondrial dysfunction and inducing reactive oxygen species [J]. *Cancer Res*, 2003, **63**(23): 8138-8144.

[50] Malhi H, Kaufman R J. Endoplasmic reticulum stress in liver disease [J]. *J Hepatol*, 2011, **54**(4): 795-809.

[51] Wang M, Kaufman R J. The impact of the endoplasmic reticulum protein-folding environment on cancer development [J]. *Nat Rev Cancer*, 2014, **14**(9): 581-597.

[52] Malhotra J D, Kaufman R J. Endoplasmic reticulum stress and oxidative stress: A vicious cycle or a double-edged sword? [J]. *Antioxid Redox Signal*, 2007, **9**(12): 2277-2293.

[53] Gao H J, Zhu Y M, He W H, *et al*. Endoplasmic reticulum stress induced by oxidative stress in decidual cells: A possible mechanism of early pregnancy loss [J]. *Mol Biol Rep*, 2012, **39**(9): 9179-9186.

[54] Corwin W L, Baust J M, Baust J G, *et al*. The unfolded protein response in human corneal endothelial cells following hypothermic storage: Implications of a novel stress pathway [J]. *Cryobiology*, 2011, **63**(1): 46-55.

[55] Yoshida H, Okada T, Haze K, *et al*. Endoplasmic reticulum stress-induced formation of transcription factor complex ERSF including NF-Y (CBF) and activating transcription factors 6alpha and 6beta that activates the mammalian unfolded protein response [J]. *Mol Cell Biol*, 2001, **21**(4): 1239-1248.

[56] Hu P, Han Z, Couvillon A D, *et al*. Autocrine tumor necrosis factor alpha links endoplasmic reticulum stress to the membrane death receptor pathway through IRE1alpha-mediated NF-kappab activation and down-regulation of traf2 expression [J]. *Mol Cell Biol*, 2006, **26**(8): 3071-3084.

[57] Do W, Herrera C, Mighty J, *et al*. Sigma 1 receptor plays a prominent role in IL-24-induced cancer-specific apoptosis [J]. *Biochem Biophys Res Commun*, 2013, **439**(2): 215-220.

[58] Li J, Shi L, Zhang X, *et al*. Recombinant adenovirus IL-24-Bax promotes apoptosis of hepatocellular carcinoma cells *in vitro* and *in vivo* [J]. *Cancer Gene Ther*, 2010, **17**(11): 771-779.

[59] Dash R, Richards J E, Su Z Z, *et al*. Mechanism by which MCL-1 regulates cancer-specific apoptosis triggered by mda-7/IL-24, an IL-10-related cytokine [J]. *Cancer Res*, 2010, **70**(12): 5034-5045.

[60] Collina S, Gaggeri R, Marra A, *et al*. Sigma receptor modulators: A patent review [J]. *Expert Opin Ther Pat*, 2013, **23**(5): 597-613.

[61] He B, Huang X, Liu X, *et al*. Cancer targeting gene-virotherapy for pancreatic cancer using oncolytic adenovirus ZD55-IL-24 in immune-competent mice [J]. *Mol Biol Rep*, 2013, **40**(9): 5397-5405.

[62] Panneerselvam J, Munshi A, Ramesh R. Molecular targets and signaling pathways regulated by interleukin(IL)-24 in mediating its antitumor activities [J]. *J Mol Signal*, 2013, **8**(1): 15.

[63] Fels D R, Koumenis C. The PERK/EIF2alpha/ATF4 module of the UPR in hypoxia resistance and tumor growth [J]. *Cancer Biol Ther*, 2006, **5**(7): 723-728.

[64] Fritsch R M, Schneider G, Saur D, *et al*. Translational repression of MCL-1 couples stress-induced EIF2 alpha phosphorylation to mitochondrial apoptosis initiation [J]. *J Biol Chem*, 2007, **282**(31): 22551-22562.

[65] Raven J F, Koromilas A E. PERK and PKR: Old kinases learn new tricks [J]. *Cell Cycle*, 2008, **7**(9): 1146-1150.

[66] Lebedeva I V, Sarkar D, Su Z Z, *et al*. Bcl-2 and Bcl-x(L) differentially protect human prostate cancer cells from induction of apoptosis by melanoma differentiation associated gene-7, mda-7/IL-24 [J]. *Oncogene*, 2003, **22**(54): 8758-8773.

[67] Pinton P, Ferrari D, Magalhaes P, *et al*. Reduced loading of intracellular Ca^{2+} stores and downregulation of capacitative Ca^{2+} influx in Bcl-2-overexpressing cells [J]. *J Cell Biol*, 2000, **148**(5): 857-862.

[68] Oakes S A, Scorrano L, Opferman J T, *et al*. Proapoptotic Bax and Bak regulate the type 1 inositol trisphosphate receptor and calcium leak from the endoplasmic reticulum [J]. *Proc Natl Acad Sci USA*, 2005, **102**(1): 105-110.

[69] Malhotra J D, Kaufman R J. ER stress and its functional link to mitochondria: Role in cell survival and death [J]. *Cold Spring Harb Perspect Biol*, 2011, **3**(9): a004424.

[70] Bhutia S K, Das S K, Azab B, *et al*. Autophagy switches to apoptosis in prostate cancer cells infected with melanoma differentiation associated gene-7/interleukin-24 (mda-7/IL-24) [J]. *Autophagy*, 2011, **7**(9): 1076-1077.

[71] Lin C H, Shih C H, Tseng C C, *et al*. CXCl12 induces connective tissue growth factor expression in human lung fibroblasts through the Rac1/ERK, JNK, and AP-1 pathways [J]. *PLoS One*, 2014, **9**(8): e104746.

[72] Buzas K, Oppenheim J J, Zack Howard O M. Myeloid cells migrate in response to IL-24 [J]. *Cytokine*, 2011, **55**(3): 429-434.

[73] You L, He B, Xu Z, *et al.* An anti-Wnt-2 monoclonal antibody induces apoptosis in malignant melanoma cells and inhibits tumor growth [J]. *Cancer Res*, 2004, **64**(15): 5385-5389.

[74] Moretti R M, Montagnani Marelli M, Mai S, *et al*. Clusterin isoforms differentially affect growth and motility of prostate cells: Possible implications in prostate tumorigenesis [J]. *Cancer Res*, 2007, **67**(21): 10325-10333.

[75] Scaltriti M, Brausi M, Amorosi A, *et al*. Clusterin (SGP-2, ApoJ) expression is downregulated in low- and high-grade

human prostate cancer [J]. *Int J Cancer*, 2004, **108**(1): 23-30.

[76] Scaltriti M, Santamaria A, Paciucci R, *et al*. Intracellular clusterin induces G2-M phase arrest and cell death in PC-3 prostate cancer cells [J]. *Cancer Res*, 2004, **64**(17): 6174-6182.

[77] Shannan B, Seifert M, Leskov K, *et al*. Challenge and promise: Roles for clusterin in pathogenesis, progression and therapy of cancer [J]. *Cell Death Differ*, 2006, **13**(1): 12-19.

[78] Trougakos I P, Lourda M, Agiostratidou G, *et al*. Differential effects of clusterin/apolipoprotein J on cellular growth and survival [J]. *Free Radic Biol Med*, 2005, **38**(4): 436-449.

[79] Bhutia S K, Das S K, Kegelman T P, *et al*. Mda-7/IL-24 differentially regulates soluble and nuclear clusterin in prostate cancer [J]. *J Cell Physiol*, 2012, **227**(5): 1805-1813.

[80] Binet F, Mawambo G, Sitaras N, *et al*. Neuronal ER stress impedes myeloid-cell-induced vascular regeneration through IRE1alpha degradation of netrin-1[J]. *Cell Metab*, 2013, **17**(3): 353-371.

[81] Pelicano H, Carney D, Huang P. ROS stress in cancer cells and therapeutic implications [J]. *Drug Resist Updat*, 2004, **7**(2): 97-110.

[82] Jiang G, Liu Y Q, Wei Z P, *et al*. Enhanced anti-tumor activity by the combination of a conditionally replicating adenovirus mediated interleukin-24 and dacarbazine against melanoma cells via induction of apoptosis [J]. *Cancer Lett*, 2010, **294**(2): 220-228.

[83] Gupta P, Emdad L, Lebedeva I V, *et al*. Targeted combinatorial therapy of non-small cell lung carcinoma using a GST-fusion protein of full-length or truncated mda-7/IL-24 with tarceva [J]. *J Cell Physiol*, 2008, **215**(3): 827-836.

[84] Fisher P B. Is mda-7/IL-24 a “magic bullet” for cancer? [J]. *Cancer Res*, 2005, **65**(22): 10128-10138.

[85] Sarkar S, Azab B, Quinn B A, *et al*. Chemoprevention gene therapy (CGT) of pancreatic cancer using perillyl alcohol and a novel chimeric serotype cancer terminator virus [J]. *Curr Mol Med*, 2014, **14**(1): 125-140.

□