Crosstalk of EDA-A2/XEDAR in the p53 Signaling Pathway

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Abstract

We recently identified X-linked ectodermal dysplasia receptor (XEDAR, also known as TNFRSF27 or EDA2R) as a direct p53 target that was frequently downregulated in colorectal cancer tissues due to its epigenetic alterations or through the p53 gene mutations. However, the role of the posttranslational regulation of XEDAR protein in colorectal carcinogenesis was not well clarified thus far. Here, we report that the extracellular NH2 terminus of XEDAR protein was cleaved by a metalloprotease and released into culture media. The remaining COOH-terminal membrane-anchored fragment was rapidly degraded through the ubiquitin-proteasome pathway. Interestingly, ectopic p53 expression also transactivated an XEDAR ligand, EDA-A2, together with XEDAR. Moreover, EDA-A2 blocked the cleavage of XEDAR and subsequently inhibited cell growth. We also found a missense mutation of the XEDAR gene in NCI-H716 colorectal cancer cells, which caused the translocation of XEDAR protein from cell membrane to cytoplasm. This mutation attenuated the growth-suppressive effect of XEDAR, indicating that membrane localization is critical for physiologic XEDAR function. Thus, our findings clearly revealed the crucial role of EDA-A2/XEDAR interaction in the p53-signaling pathway.

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Introduction

Mutations of the p53 tumor suppressor gene are the most frequent alterations observed in human cancer cells (1-3). The majority of the p53 gene mutations occurs within the DNA binding region of the protein and affects its site-specific DNA binding activity. Therefore, the most significant function of p53 is attributed to the sequence-specific transcription factor, and identification of p53 target genes is essential to fully clarify the role of p53 in human carcinogenesis. Until now, we have reported several novel p53 target genes, such as p53R2, p53AIP1, p53RDL1, and PADI4 (4-11), and showed the critical mechanism by which p53 inhibits malignant transformation.

Due to the inactivation of p53, expressions of p53 target genes are expected to be altered in cancer tissues. To identify a p53 target gene(s) that is indispensable for p53-dependent tumor suppression, we screened two data sets of genome-wide expression profile; one was obtained using the cells in which wild-type p53 was exogenously introduced (12) and the other was obtained using >1,000 clinical cancer cases (13-19). The analysis of these two data sets identified CDC20 as a p53-suppressive gene that is highly expressed in nearly half of various cancer tissues (20). In addition, we recently reported that X-linked ectodermal dysplasia receptor (XEDAR, also known as TNFRSF27 and EDA2R) is a direct p53 target that mediates p53-induced anoikis pathway (21). XEDAR expression was decreased in about two thirds of colorectal cancer tissues due to its epigenetic alterations as well as the p53 gene mutations. Furthermore, we found mutations of the XEDAR gene in one colorectal cancer tissue and one cell line, although its frequency is very low. Thus, XEDAR is likely to function as a colorectal tumor suppressor.

XEDAR belongs to a member of tumor necrosis factor receptor superfamily that is involved in various signaling pathways including immune response, inflammation, development, and carcinogenesis. Although it lacks a discernable death domain, XEDAR was shown to promote apoptotic signaling through the binding of its ligand EDA-A2 (22). EDA-A2 is highly expressed in embryonic tissues, and its germline mutations associate with impairment of skin appendages, such as sweat glands and hair follicles (23, 24). However, the role of EDA-A2 in carcinogenesis has not been discussed yet. Our previous analysis revealed that XEDAR regulated the apoptotic pathway through the interaction with and stabilization of FAS, a key regulator of apoptotic signaling (25). Both FAS and its ligand FASL were shown to be transactivated by p53 and promote apoptotic pathway (26). In this study, we examined the possible involvement of EDA-A2 in a p53-mediated apoptosis.
**Materials and Methods**

**Plasmid construction**

Plasmid expressing HA-tagged ubiquitin was kindly provided by Dr. Tomohiko Ohta (St. Marianna University School of Medicine, Kawasaki, Japan). Y8H-mutant (Y6H), L99I-mutant (L99I), L122I-mutant (L122I), and L124I-mutant (L124I) XEDAR were generated using the QuickChange site-directed mutagenesis kit (Stratagene). Primers used in this study are shown in Supplementary Table S1.

**Recombinant Fc-EDA-A2 and Fc-FASL**

pCAGGS/Fc-EDA-A2 and pCAGGS/Fc-FASL expression plasmids were designed and constructed as follows. A series of coding sequence of the trypsinogen A signal sequence (amino acids of 1-20), the Fc portion of human IgG1 (amino acids 101-330 of Genbank accession number P01857.1), a linker sequence (EFCRYPAQWRP), and predicted soluble form of EDA-A2 (amino acids 160-389; ref. 27) or FASL (amino acids 139-281) were PCR amplified and subcloned into pCAGGS expression plasmid. Primers are shown in Supplementary Table S1. Life Technologies FreeStyle 293F Cells (Invitrogen) were transfected using FuGENE6 (Roche) according to the manufacturer’s protocol. Recombinant protein was purified from culture media by affinity on protein A-Sepharose.

**Cell culture and transfections**

HEK293, HEK293T, U373MG, H1299, NCI-H716, and NHDF cell lines were purchased from the American Type Culture Collection (28). Life Technologies FreeStyle 293F Cells were purchased from Invitrogen. All cells were cultured under conditions recommended by their respective depositors. Replication-deficient recombinant viruses Ad-p53 or Ad-LacZ expressing p53 or LacZ, respectively, were generated and purified as previously described (4). U373MG cells were infected with viral solutions at various multiplicities of infection and incubated at 37°C until the time of harvest. For treatment with genotoxic stress, NHDF cells were continuously incubated with Adriamycin for 48 hours as indicated in the figure legend. Cells were transfected with plasmids using FuGENE6 (Roche) or Lipofectamine LTX with Plus Reagent (Invitrogen). Small interfering RNA oligonucleotides, commercially synthesized by Sigma Genosis, were transfected with the Lipofectamine 2000 reagent (Invitrogen) for 4 hours. Sequences for each oligonucleotide were indicated in Supplementary Table S1. Life Technologies FreeStyle 293F Cells were purchased from Invitrogen. All cells were cultured under conditions recommended by their respective depositors. Replication-deficient recombinant viruses Ad-p53 or Ad-LacZ expressing p53 or LacZ, respectively, were generated and purified as previously described (4). U373MG cells were infected with viral solutions at various multiplicities of infection and incubated at 37°C until the time of harvest. For treatment with genotoxic stress, NHDF cells were continuously incubated with Adriamycin for 48 hours as indicated in the figure legend. Cells were transfected with plasmids using FuGENE6 (Roche) or Lipofectamine LTX with Plus Reagent (Invitrogen). Small interfering RNA oligonucleotides, commercially synthesized by Sigma Genosis, were transfected with the Lipofectamine 2000 reagent (Invitrogen) for 4 hours. Sequences for each oligonucleotide were indicated in Supplementary Table S1. For suspension culture, six-well plates were coated with 2 mL of poly-2-hydroxyethyl methacrylate (poly-HEMA; Sigma) solution in ethanol (10 mg/mL) for at least 3 days until the solvent had completely evaporated. Cells were suspended in 2 mL of media containing 0.5% methylcellulose and plated onto poly-HEMA-coated dishes. For the analysis of proteasomal degradation or ectodomain shedding of XEDAR protein, cells were treated with proteasome inhibitor MG132 (Calbiochem) or metalloproteinase inhibitor GM6001 (Calbiochem) before harvest. Cell viability was determined by MTT assay using Cell Counting Kit-8 (Dojindo). Cell death was measured by trypan blue (Invitrogen) uptake.

**Quantitative real-time PCR and semiquantitative reverse transcription-PCR**

Isolation of total RNA from cultured cells was done using RNeasy spin column kits (Qiagen) according to the manufacturer’s instructions. cDNAs were synthesized with the SuperScript Preamplification System (Invitrogen). Quantitative real-time PCR was conducted with the SYBR Green I Master on a LightCycler 480 (Roche) according to the manufacturer’s recommendations. Each experiment was done in duplicate. β2-Microglobulin was used for normalization of expression levels. Semiquantitative reverse transcription-PCR was conducted as previously described (7). PCR products were separated on 15% acrylamide gel. Primer sequences were indicated in Supplementary Table S1.

**Antibodies**

Rabbits were immunized with the recombinant proteins corresponding to the extracellular domain (amino acids 1-139) or intracellular domain (amino acids 165-297) of XEDAR. Antibodies against the extracellular domain (anti-XEDARn antibody) or intracellular domain (anti-XEDARc antibody) of XEDAR were subsequently purified on antigen affinity columns. Anti–β-actin monoclonal antibody (clone AC15) was purchased from Sigma. Anti-XEDAR polyclonal antibody (T-14), anti-HSP90 monoclonal antibody (F-8), anti–c-Myc monoclonal (9E10) and polyclonal (A-14) antibody, and anti-HA polyclonal (Y-11) antibody were purchased from Santa Cruz Biotechnology. Anti–EDA-A2 polyclonal antibody (AF922) was purchased from R&D Systems.

**Western blot analysis**

To prepare whole-cell lysates, cells were collected and lysed in chilled HBST buffer (10 mmol/L HEPES at pH 7.4, 150 mmol/L sodium chloride, and 0.5% TritonX-100) for 30 minutes on ice and centrifuged at 16,000 × g for 15 minutes. To concentrate the media, media were mixed with equal volume of acetone and kept at −80°C for 1 hour. Then, mixture was centrifuged at 16,000 × g for 15 minutes, and the resulting protein pellets were dissolved in SDS sample buffer. For the subcellular fractionation, cells were detached with a scraper and lysed in 20 mmol/L HEPES buffer (pH 7.5) containing 250 mmol/L sucrose. The cell homogenate was centrifuged at 1,000 × g for 7 minutes. The supernatant was centrifuged at 12,000 × g for 30 minutes to remove mitochondria, endosomes, and lysosomes. The supernatant was further centrifuged at 105,000 × g for 60 minutes, and the resulting pellet was used as the microsomal fraction. Samples were subjected to SDS-PAGE and immunoblotting by a standard procedure. Bands were quantified by the Image J software.
Immunocytochemistry

Immunocytochemistry was done as previously described (4). For cell surface staining, cells were labeled under nonpermeabilized conditions using the buffer without triton X-100.

Results

Role of EDA-A2 in the XEDAR pathway

EDA-A1 and EDA-A2 are two major isoforms of the EDA gene, which differ only by a six-base insertion/deletion. To examine the possible regulation of EDA by p53, we quantitatively analyzed the expression of EDA in the p53-introduced U373MG cells. Consequently, we found that EDA mRNA was significantly increased by an ectopic expression of p53 (Fig. 1A). Electrophoresis of PCR products revealed that both EDA-A1 and EDA-A2 transcripts were induced by p53 (Fig. 1A), indicating that EDA-A2/ XEDAR signaling was regulated by p53. Then, we treated NHDF cells with Adriamycin and found the dose-dependent induction of EDA mRNA (Supplementary Fig. S1A). Additionally, knockdown of p53 expression by siRNA remarkably suppressed EDA expression (Supplementary Fig. S1B). These findings clearly revealed that EDA expression was regulated by endogenous p53.

Anti-XEDAR antibody detected three protein bands of approximately 55, 48, and 35 kDa, which were decreased by treatment with siRNA-targeting XEDAR (Supplementary Fig. S1C; Fig. 1B). These three bands were also detectable in pcDNA3.1/XEDAR-transfected cells (Fig. 1B). Interestingly, when we transfected HEK293 cells with

**FIGURE 1.** Roles of EDA-A2 in the p53-XEDAR signaling. A, quantitative PCR analysis of EDA transcript in U373MG cells 48 h after infection with Ad-p53 or Ad-LacZ at the indicated multiplicity of infection (MOI; left). Semiquantitative reverse transcription-PCR analysis in U373MG cells 48 h after infection with Ad-p53 or Ad-LacZ at 40 multiplicity of infection (right). PCR products were separated on 15% acrylamide gel. cDNA clone was used as a template for amplification in lane 4 (EDA-A1) and 5 (EDA-A2). β2-Microglobulin (B2M) was used for the normalization of expression levels. B, expression of XEDAR protein in HEK293 cells after transfection with each siRNA or expression plasmid. Black arrowheads, three major bands of XEDAR protein (55, 48, and 35 kDa). Open arrowheads, nonspecific bands. The results of β-actin and HSP90 were indicated as control. C, expression of the XEDAR protein in HEK293 cells after transfection with the XEDAR expression plasmid together with either EDA-A2 or mock plasmid. β-Actin was used for the normalization of expression levels. D, expression of the XEDAR protein in HEK293 cells incubated with 1 μg/mL of recombinant EDA-A2 protein (Fc-EDA-A2) for 24 h. β-Actin was used for the normalization of expression levels.
EDA-A2 expression plasmid, the 55- and 35-kDa bands were drastically decreased, whereas the 48-kDa band was slightly increased (Fig. 1C). We constructed plasmid designed to express recombinant EDA-A2 protein fused to the Fc region of human IgG1 (pCAGGS/Fc-EDA-A2; ref. 29) and purified recombinant protein from the culture media of pCAGGS/Fc-EDA-A2 transfected cells. Treatment of HEK293 cells with 1 μg/mL of recombinant Fc-EDA-A2 also reduced the intensity of 55- and 35-kDa bands of endogenous XEDAR (Fig. 1D). Taken together, ligand binding would alter the property of XEDAR protein.

**Ectodomain shedding of XEDAR**

*XEDAR* encodes a type I transmembrane protein including a single transmembrane domain with a NH2-terminal extracellular domain and a COOH-terminal intracellular domain. When we analyzed cell extracts from XEDAR-introduced HEK293 cells by Western blotting, the 35-kDa band was recognized by an antibody raised against the COOH terminus of XEDAR (anti-XEDARc antibody) but not by an antibody against the NH2 terminus of XEDAR (anti-XEDARn antibody; Fig. 2A), whereas 55- and 48-kDa bands were recognized with both antibodies. In contrast, a 20-kDa fragment was detectable with the anti-XEDARn antibody in the concentrated culture media but not in the cell extracts (Fig. 2A). These findings suggested that the 20-kDa of NH2-terminal extracellular fragment of XEDAR would be cleaved and released into the culture media.

Metalloproteinases are highly expressed in cancer tissues, and promote tumor invasion and metastasis (30, 31). Our results supported these findings and suggested that XEDAR might be a target for cancer therapy. **FIGURE 2.** Ectodomain shedding of XEDAR. A, 24 h after transfection with each plasmid, HEK293 cells were grown in serum-free media for 12 h. The acetone-precipitated media and the cell lysates were subjected to immunoblotting (IB) with anti-XEDARn and anti-XEDARc antibody. W.C.L, whole-cell lysate. B, 4 h after transfection with the XEDAR expression plasmid, the cells were grown in serum-free media containing 0 to 40 μmol/L of GM6001, a broad-spectrum metalloproteinase inhibitor, for 20 h. The media were concentrated by acetone precipitation and subjected to immunoblotting with anti-XEDARn antibody. C, 24 h after transfection with XEDAR expression plasmid, HEK293 cells were incubated in the conditioned media that were used for the culture of HEK293T cells transfected with pCAGGS/Fc-EDA-A2, or pCAGGS/Fc-FASL plasmid or mock plasmid. Sixteen hours after the incubation, media were concentrated and subjected to immunoblotting with the anti-XEDARn antibody. D, HEK293 and NCI-H716 cells were transfected with each plasmid, and 24 h after transfection, the same numbers of cells were seeded in culture plates coated with poly-HEMA. After 48 h of incubation, cell viability was measured by MTT assay. Results are given as a ratio against the cells transfected with mock plasmid. *, *P < 0.05 by Student’s t test. Right, the expression of EDA in each cell. β-Actin was used for the normalization of expression levels.
previous result indicated that the inactivation of XEDAR conferred anoikis resistance that was associated with the metastatic property of cancer cells. Therefore, we considered that metalloproteinases would promote tumor metastasis through XEDAR cleavage. To examine this hypothesis, we added broad-spectrum metalloproteinase inhibitor GM6001 to the culture media and found that the amount of NH$_2$-terminal fragment in the culture media was decreased in a GM6001 dose-dependent manner (Fig. 2B). Thus, XEDAR was shown to undergo processing by metalloproteinase at its extracellular domain, namely ectodomain shedding.

To further characterize the metalloproteinases-mediated ectodomain shedding, we surveyed the potential cleavage sites by using the software program ProfileDB (http://www.proteolysis.org/proteases/m_goto_profiledb). Consequently, three possible cleavage sites (G98_L99, Q121_L122, and S123_L124) were predicted. Subsequently, we constructed plasmid-expressing mutant forms of XEDAR (L99I, L122I, and L124I). When we transfected HEK293 cells with these plasmids, the cleaved forms of XEDAR (35 and 20 kDa) were remarkably decreased in cells expressing L122I-mutant XEDAR protein (Supplementary Fig. S2), whereas L99I and L124I mutations indicated no or a marginal effect. In addition, L122I-mutant XEDAR exhibited an enhanced growth-inhibitory effect than wild-type XEDAR (Supplementary Fig. S2B), probably due to the defect of ectodomain shedding by metalloproteinases. Because the amount of 35-kDa protein remarkably decreased in the presence of EDA-A2 (Fig. 1C and D), we considered that ligand binding might inhibit the processing of XEDAR protein. When XEDAR-transfected HEK293 cells were incubated in conditioned media that were used for culturing the Fc-EDA-A2-transfected HEK293T cells, the cleavage of the NH$_2$-terminal fragment of XEDAR was completely blocked (Fig. 2C). Moreover, the result of MTT assay revealed that ectopic expression of EDA-A2 suppressed the growth of HEK293 cells, whereas that of EDA-A2 had no effect on NCI-H716 colorectal cancer cells that carry the XEDAR mutation (Supplementary Fig. S2C; Fig. 2D). Taken together, our findings indicated that a metalloproteinase

**FIGURE 3.** Ubiquitin-proteasomal degradation of XEDAR. A, HEK293 cells were incubated with MG132 for 5 h before harvest. DMSO was used as control. β-Actin was used for normalization of expression levels. B, HEK293 cells transfected with XEDAR expression plasmid were incubated with MG132 for 5 h before harvest. DMSO was used as control. β-Actin was used for normalization of expression levels. C, expression of the XEDAR protein in HEK293 cells incubated with 0.1 μg/mL of recombinant EDA-A2 protein for 10 h. MG132 (10 μmol/L) was added for 5 h before harvest. Cell lysates were immunoprecipitated (IP) using anti-Myc antibody, followed by immunoblotting with anti-HA antibody.
cleaved XEDAR at the extracellular domain and generated a soluble 20-kDa fragment and a membrane-anchored 35-kDa fragment. However, EDA-A2 binding blocked the processing of XEDAR and subsequently suppressed cell growth.

Interestingly, Fc-FASL treatment partially inhibited the cleavage of XEDAR protein (Fig. 2C). This result is concordant with our previous finding that FAS-XEDAR interaction increased protein stability. Our findings would partially explain the molecular mechanisms underlying the coactivation of XEDAR and FAS signaling in the p53 downstream pathway.

**Ubiquitin-proteasomal degradation of XEDAR**

To elucidate the physiologic significance of XEDAR processing, we treated HEK293 cells with MG132, a 26S proteasome inhibitor. Consequently, we found that the endogenous 35-kDa XEDAR fragment was accumulated...
in a dose-dependent manner (Fig. 3A). Similarly, the accumulation of 35-kDa fragment was observed in XEDAR-introduced HEK293 cells that were treated with MG132 (Fig. 3B). On the other hand, when we blocked the ectodomain shedding of XEDAR with Fc-EDA-A2, MG132 treatment did not increase the expression of the 35-kDa form of XEDAR protein (Fig. 3C).

We then investigated the ubiquitination of XEDAR protein. HEK293T cells were transfected with plasmids expressing HA-ubiquitin and/or Myc-XEDAR, followed by the immunoprecipitation with anti-Myc antibody. The result of the Western blotting with the anti-HA antibody revealed that XEDAR protein was multiply conjugated with ubiquitin (Fig. 3D), indicating that XEDAR undergoes polyubiquitination in vivo. Our findings implied that ectodomain shedding of XEDAR protein by metalloproteinase resulted in the ubiquitin-proteasomal degradation of COOH-terminal membrane-anchored fragment.

**XEDAR mutation caused abnormal membrane trafficking**

We previously identified a missense mutation in the XEDAR gene (exon 2 T74C; Y8H) in one colorectal cancer cell line (NCI-H716; ref. 25). To investigate the biological significance of this missense mutation, we constructed the plasmid-expressing mutant form of XEDAR (pcDNA3.1/Y8H-XEDAR). We transfected H1299 cells with plasmid-expressing wild-type (wt-) or Y8H-XEDAR, and found that wt-XEDAR protein generally localized at the plasma membrane, whereas Y8H-XEDAR protein was retained within the cytoplasm (Fig. 4A). In the absence of membrane permeabilization with triton X-100, the cells expressing Y8H-XEDAR were hardly stained with the antibody against the NH₂-terminal extracellular domain of XEDAR, whereas the cells expressing wt-XEDAR were clearly stained (Fig. 4A). These data indicated that the mutation at the tyrosine residue caused the abnormal membrane trafficking of XEDAR protein.

To further clarify the mechanism by which XEDAR mutation altered its subcellular localization, we conducted Western blotting using HEK293 cells that were transfected with wt-XEDAR or Y8H-XEDAR expression plasmid. Consequently, we found that 55 and 35-kDa forms of XEDAR were remarkably decreased in cells transfected with Y8H-XEDAR expression plasmid (Fig. 4B). Similarly, 55 and 35-kDa forms of XEDAR protein were absent in NCI-H716 cells that expressed the Y8H-XEDAR protein (Fig. 4C). Furthermore, when cell extracts from XEDAR-transfected HEK293 cells were fractionated by differential centrifugation, the 55-kDa form of XEDAR was dominantly accumulated in microsomal fraction in which membrane proteins were enriched, whereas the amount of 48-kDa form of XEDAR was decreased in this fraction (Fig. 4B). Coupled with the result of immunocytochemistry, our findings suggest that possible posttranslational modification would alter the molecular weight of XEDAR protein from 48 to 55 kDa. This modification would induce the translocation of XEDAR protein from the cytoplasm (48-kDa form) to the plasma membrane (55-kDa form); however, Y8H mutation attenuated this modification and subsequently inhibited the membrane localization of XEDAR protein.

Because our previous findings revealed the crucial role of XEDAR in anoikis, we examined the effect of XEDAR...
Discussion

The EDA gene, encoding an XEDAR ligand, was found to be mutated in patients with hypohidrotic ectodermal dysplasia, but its involvement in carcinogenesis was not reported previously. FAS/FASL pathway and TRAIL/TRAIL receptor (DR5) pathway were shown to be regulated by p53 (33). Likewise, we here showed that both EDA-A2 and XEDAR were induced by p53. We also found that XEDAR was cleaved at its extracellular domain by metallocproteinase, and EDA-A2 blocked this cleavage processing of XEDAR (Fig. 5). The physiologic significance of ectodomain shedding varies between substrate proteins. Tumor necrosis factor α is cleaved by TACE and exhibits strong systemic effects (34). In contrast, E-cadherin is cleaved by metallocproteinase, and a residual membrane-tethered product is degraded by intracellular proteolytic pathway (35). Our data indicated that ectodomain shedding resulted in the degradation of the residual XEDAR protein by ubiquitin-proteasome pathway, similar to E-cadherin.

XEDAR was previously shown to be deubiquitinated by CYLD (36). Loss-of-function mutations in the CYLD gene were associated with predisposition to skin appendices tumors, called cylindromatosis (37). Because XEDAR is highly expressed in epidermal tissues, CYLD mutations are likely to cause the decreased expression of XEDAR. In addition, CYLD mRNA was also shown to be decreased in colorectal cancer tissues (38). Therefore, deregulation of XEDAR by CYLD inactivation would partially explain the pathogenesis of colorectal cancer as well as familial cylindromatosis.

Furthermore, we found a loss-of-function mutation of the XEDAR gene in the colorectal cancer cell line NCI-H716. Among 20 colorectal cancer cell lines examined, only NCI-H716 is a suspension cell line that acquires anchorage independency (21). These findings are concordant with our previous findings that XEDAR is a key mediator of anokis. When we compared the amino acid sequences of XEDAR among several species, the eighth tyrosine residue of XEDAR protein is conserved from chick to human, suggesting that this amino acid exerts some biological effect (Supplementary Fig. S3). Interestingly, the eighth tyrosine residue was predicted to be a possible O-sulfation site by the Sulfinator program (http://www.expasy.ch/tools/sulfinator/) in all species (Supplementary Fig. S3; ref. 39). Because accumulated evidence indicated the role of O-sulfation in protein secretion, protein-protein interaction, or ligand-receptor binding (40), our findings suggested the possible regulatory mechanism of XEDAR function through O-sulfation.

We have previously revealed that XEDAR was frequently suppressed in colorectal cancer tissues through its epigenetic alterations or p53 mutations. In this study, we identified the additional mechanism of the XEDAR inactivation through the ectodomain shedding and subsequent proteasomal degradation. Interestingly, this XEDAR processing was inhibited by the binding of EDA-A2 to XEDAR, and subsequently, the cell growth was suppressed. Although the physiologic importance of XEDAR or its ligand EDA-A2 and their contribution to antitumor response or cancer therapy in vivo remain to be investigated, our findings implied the possible role of EDA-A2/XEDAR interaction in the p53-signaling pathway as a barrier against tumor development and progression.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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