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Epigenetic regulation of the transcription factor Foxa2 directs differential elafin expression in melanocytes and melanoma cells

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ABSTRACT

Elafin, a serine protease inhibitor, induces the intrinsic apoptotic pathway in human melanoma cells, where its expression is transcriptionally silenced. However, it remains unknown how the elafin gene is repressed in melanoma cells. We here demonstrate that elafin expression is modulated *via* epigenetically regulated expression of the transcription factor Foxa2. Treatment of melanoma cells with a DNA methyltransferase inhibitor induced elafin expression, which was specifically responsible for reduced proliferation and increased apoptosis. Suppression of Foxa2 transcription, mediated by DNA hypermethylation in its promoter region, was released in melanoma cells upon treatment with the demethylating agent. Luciferase reporter assays indicated that the Foxa2 binding site in the elafin promoter was critical for the activation of the promoter. Chromatin immunoprecipitation assays further showed that Foxa2 bound to the elafin promoter *in vivo*. Analyses of melanoma cells with varied levels of Foxa2 revealed a correlated expression between Foxa2 and elafin and the ability of Foxa2 to induce apoptosis. Our results collectively suggest that, in melanoma cells, Foxa2 expression is silenced and therefore elafin is maintained unexpressed to facilitate cell proliferation in the disease melanoma.

1. Introduction

Elafin, also known as skin-derived antileukoproteinase (SKALP) or peptidase inhibitor 3 (PI3), is an inhibitor targeting serine proteases, including human neutrophil elastase, with documented antimicrobial and anti-inflammatory functions [1,2]. Initial studies have associated elafin with inflammation, and subsequent investigations have shown differential expression of elafin in many human malignancies [3] and association of its expression with overall survival in some cancer types [3–5]. We have recently shown that elafin induces apoptosis in human melanoma cells through the p53-dependent intrinsic pathway, and expression of the protein is repressed in human melanoma tissue specimens and cell lines, but not normal melanocytes, suggesting that silencing of elafin expression in melanoma contributes to progression of the disease [6]. However, the underlying mechanism of elafin suppression in melanoma remains to be established.

Chromosomal aberrations in the region encompassing the elafin gene, as exemplified by amplification in ovarian and breast cancers [7,8] and deletion in oral squamous cell carcinoma [9], may confer cancer- or tissue-specific expression patterns. High-degree polymorphism of the elafin promoter sequence [10] may promote the differential binding of transcription factors in various tissues. At the molecular level, a number of transcription factors play roles in elafin expression, implying complex transcriptional regulation of the elafin promoter. Alterations in the activating/inactivating ratio of the CCAAT/enhancer binding protein β (C/EBPβ) isoforms, leading to their differential availability for binding to the elafin promoter, confer variations in elafin expression patterns between normal and breast cancer cells [11]. Elafin expression is activated by inflammatory cytokines through binding of nuclear factor-κB (NF-κB) to its promoter region in ovarian cancer cells [5] and pulmonary epithelial cells [12]. Induction of Ap1 in breast cancer cells

Abbreviations: Foxa2, a member of subclass A of the forkhead box containing transcription factor family; SKALP, skin-derived antileukoproteinase; PI3, peptidase inhibitor 3; C/EBPβ, CCAAT/enhancer binding protein β; RT-PCR, reverse transcription-polymerase chain reaction; MSP, methylation-specific PCR; 5-Aza-CdR, 5-aza-2′-deoxycytidine.

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leads to transactivation of the elafin gene through the Ap1 site in the promoter [13]. A quantitative chromatin immunoprecipitation screening identified the elafin promoter as a high-affinity target for c-Myc [14].

In the majority of human cancers, aberrant epigenetic regulation is associated with altered transcription of genes critical for tumor initiation and progression, and altered DNA methylation is a key component of the cancer epigenome [15]. As a member of the forkhead box containing transcription factor family, Foxa2 has been shown to play crucial roles in embryonic development and epithelial differentiation [16]. DNA methylation of the Foxa2 promoter has been reported in several human cancer types including pancreatic, thyroid, lung and breast cancers [17–20].

We show here that elafin expression is indirectly silenced in melanoma cells by methylation-mediated repression of Foxa2, and that Foxa2 induces cellular apoptosis by directing elafin expression.

2. Materials and methods

2.1. Cell lines and materials

Human melanoma cell lines and normal human epidermal melanocytes (NHEM) were described previously [6]. To construct the Foxa2-expressing plasmid pcDNA-Foxa2, the coding region of Foxa2 gene was amplified by PCR using primers 5′-ACTACGAATT CATGCTGGGAGCGGTGAAGATGG-3′ and 5′-ACTACGGATCCTCAA TGGTGATGGTGATGA-3′ and cloned into the *Eco*RI and *Xba*I sites of pcDNA3.1(+)-Myc/His (Invitrogen, Carlsbad, CA). The Foxa2-expressing lentiviral vector pLenti-Foxa2 was constructed by subcloning the Foxa2 gene (obtained by digestion of pcDNA-Foxa2 with *Sac*I/*Pme*I followed by treatment with T4 polymerase) into *Eco*RV site of pLentiM1.3hygro vector (Macrogen, Seoul, Korea). Elafin-specific siRNA (sc-42866), Foxa2-specific siRNA (sc-35569) and non-targeting control siRNA (sc-37007) were purchased from Santa Cruz (Santa Cruz, CA). Antibodies against p53, p21, Bax, Puma, Noxa, and cleaved caspase 3 were from Cell Signaling Technology (Beverly, MA). Antibodies against elafin, Foxa2, and β-actin were from Santa Cruz. Western blot analysis with cell lysates was performed as described previously [6]. Sodium bisulfite, formaldehyde, dimethyl sulfoxide (DMSO), 5-aza-2′-deoxycytidine (5-Aza-CdR), and 4′,6-diamidino-2-phenylindole (DAPI) were from Sigma (Saint Louis, MO).

2.2. Proliferation and apoptosis assays

Cell proliferation, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL), and caspase-3 activity assays were performed as described previously [6].

2.3. Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted using TRIzol reagent (Invitrogen) and used for RT-PCR with the AccessQuick RT-PCR system (Promega, Madison, WI). The 158 bp Foxa2 gene fragment was amplified with primers 5′-GAGCAGCTACTATGCAGAGC-3′ and 5′-ACGACGACAT GTTCATGGAG-3′. The 664 bp β-actin gene fragment was amplified with the primers described earlier [6]. Quantitative real-time RT-PCR was performed using total RNA from each sample and Taqman EZ RT-PCR Core Reagents (Applied Biosystems, Foster City, CA). The primers for amplification of the elafin gene were 5′-GTTTCAGT-TAAA GGTCAAGA-3′ and 5′-GTCAGTATCTTTCAAGCAGC-3′, and those for Foxa2 are specified above. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the reference gene.

2.4. Luciferase reporter assay

To generate the human elafin–luciferase fusion reporter plasmid pGR330, the 700 bp elafin promoter (−700/−1, nucleotide positions are presented relative to the start site of translation) was amplified from genomic DNA using primers 5′-ATCCAGAGCT CTATACATGATACATGTTTTC-3′ and 5′-ACTACGGATCC GGTGTCAG-GAAGGTGTTTG-3′. The PCR product was cloned into the *Sac*I and *Bgl*II sites of the pGL3-Basic vector (Promega). The reporter plasmid pGR334 was constructed by cloning the 94 bp elafin promoter product (−94/−1) amplified by PCR of pGR330 with primers 5′-ATCCAGAGCTCTAAATACCACAGACCCGCCC-3′ and 5′-ACTACGGA TCCGGTGTCAGGAAGGTGTTTG-3′, into the *Sac*I and *Bgl*II sites of pGL3-Basic. Reporter plasmids with mutations in the Foxa2 and/or C/EBPβ binding sites (pGR331, pGR332 and pGR333) were generated by site-directed mutagenesis of pGR330 using the QuikChange XL site-directed mutagenesis kit (Stratagene, La Jolla, CA). The Foxa2 binding site mutation was generated using primers 5′-GTTTTCTACTACTTTCTGATGCCGTTCTCCCTACCACTGTGATT-3′, and 5′-AATCACAGTGGTAGGGAGAACGGCATCAGAAAGTAGTAGAAAAC-3′. Mutations in the C/EBPβ binding sites were generated using the primers as described previously [11]. All the plasmid constructs were confirmed by sequencing.

Cells were transfected with a firefly luciferase reporter plasmid, along with the transfection control plasmid pRL-TK, expressing Renilla luciferase (Promega), using Lipofectamine. After 24 h, cells were treated with 5 μM 5-Aza-CdR for 48 h, and analyzed for luciferase activity. To determine the effects of Foxa2 overexpression, cells were co-transfected with each reporter plasmid plus pRL-TK, along with either pcDNA-Foxa2 or pcDNA3.1, and analyzed for luciferase activity. Firefly luciferase activity was determined as described previously [6].

2.5. Methylation-specific PCR (MSP)

Genomic DNA was isolated from cell lines using the QIAamp DNA mini Kit (Qiagen, Germantown, MD). An aliquot of genomic DNA (1 μg) was treated with sodium bisulfite using the EZ DNA Methylation-Gold Kit (Zymo Research, Orange, CA), according to the protocol recommended by the manufacturer. For MSP of the Foxa2 promoter (−354/−229), sodium bisulfite-treated DNA was amplified with primers specific for methylated (5′-TTTT CGCGTCG CGCGTTTTTCGTC-3′ and 5′-AAAACCCAAACCAAC GCCCCGCGA-3′) or unmethylated (5′-TAGTTTTTGTGTTGTGTGT TTTTTGTT-3′ and 5′-AAAAAAACCCAAACCAACACCCCACAA-3′) sequences.

2.6. Chromatin immunoprecipitation assay

A375 cells (1×10^7) were treated with 5-Aza-CdR or DMSO for 48 h. In another experimental set, cells were transiently transfected with pcDNA-Foxa2 or pcDNA3.1(+)-Myc/His for 48 h. The chromatin immunoprecipitation assay was performed using the EZ-Magna ChIPTM Kit (Millipore, Billerica, MA) according to the manufacturer's protocol. Binding of Foxa2 to the elafin promoter was accessed by PCR with primers 5′-ATACATGATA CATGTTTTCTACTAC-3′ and 5′-CCTTCCTTCTTCCCTGGTCCTGCA-3′, which were specific for the −699/−478 region in the elafin promoter encompassing the Foxa2 binding site (−668/−657). Immunoprecipitation reactions were performed in triplicate.

2.7. Statistical analysis

Proliferation, apoptosis, caspase activity and luciferase reporter assays were performed in triplicate. Data are presented as mean values ± standard deviations (STD). The Student's *t* test was used

for statistical analysis, with $p < 0.05$ defined as the level of significance.

3. Results

3.1. Elafin expression is epigenetically silenced in human melanoma cells

Previously, we showed that elafin is transcriptionally repressed in human melanoma tissues and cell lines [6]. To understand the underlying mechanism of the repression, we explored the possibility of epigenetic regulation of elafin expression, which was prompted by the earlier finding that elafin is upregulated in bladder cancer cells after 5-Aza-CdR treatment [21]. Quantitative real-time RT-PCR and Western analyses revealed that treatment of melanoma cell lines (A375, SKMEL5 and WM2664) with 5-Aza-CdR induced elafin expression (Fig. 1A, B). In contrast, the elafin level in normal human epidermal melanocytes (NHEM), expressing elafin *per se*, remained unchanged upon the treatment. Elafin induces apoptosis in human melanoma cells by mechanisms involving upregulation of p53 [6]. Consistently, 5-Aza-CdR treatment elicited a marked increase in the levels of p53 and its downstream effector molecules (p21, Bax, Puma and Noxa) in melanoma cells, but not normal melanocytes (Fig. 1B), and consequently, a significant decrease in proliferation rates (Fig. 1C) and increase in caspase-3 activity and the TUNEL-positive cell fraction (Fig. 1D, E). To define whether the induced expression of elafin was responsible for the pro-apoptotic effect of 5-Aza-CdR, cells were transiently transfected with elafin-specific or non-targeting control siRNA and followed by 5-Aza-CdR treatment. The functionality of siRNA used was verified by quantitative real-time RT-PCR analysis of elafin mRNA and Western analysis of elafin and p53 (data not shown). Reduced proliferation of melanoma cells mediated by 5-Aza-CdR was significantly, but not fully, recovered in the presence of elafin siRNA (Fig. 1C). Moreover, caspase 3 activity and the TUNEL-positive fraction of melanoma cells in the presence of 5-Aza-CdR were markedly reduced upon transfection with elafin siRNA (Fig. 1D, E). Our results indicate that elafin is differentially expressed in melanoma cells and melanocytes due to epigenetic regulation involving DNA methylation, and that expression of elafin is, to a large extent, responsible for apoptosis of melanoma cells induced by 5-Aza-CdR.

3.2. Foxa2 expression in melanoma cells is silenced by promoter hypermethylation

No CpG islands were identified in the promoter region of the elafin gene with the CpG island prediction software programs, CpG Island Searcher (cpgislands.usc.edu) and CpG Islands (www.ualberta.ca/~stothard/javascript/cpg_islands.html), implying modulation of elafin expression through DNA methylation of upstream regulators. Several putative binding sites for transcription factors in the elafin promoter were predicted with the TFSE ARCH Ver1.3 program (www.cbrc.jp/research/db/TFSE ARCH.html). Among these predicted binding sites, we identified a putative consensus sequence for Foxa2 (ATTATTTTCTCC) between positions −668 and −657 of the promoter. RT-PCR and Western analyses revealed barely detectable levels of Foxa2 mRNA and protein in melanoma cells, respectively, compared to expression in normal melanocytes (Fig. 2A), indicative of Foxa2 silencing at the transcriptional level in melanoma. Quantitative real-time RT-PCR analyses showed that 5-Aza-CdR induced a ~2- to 4-fold increase in

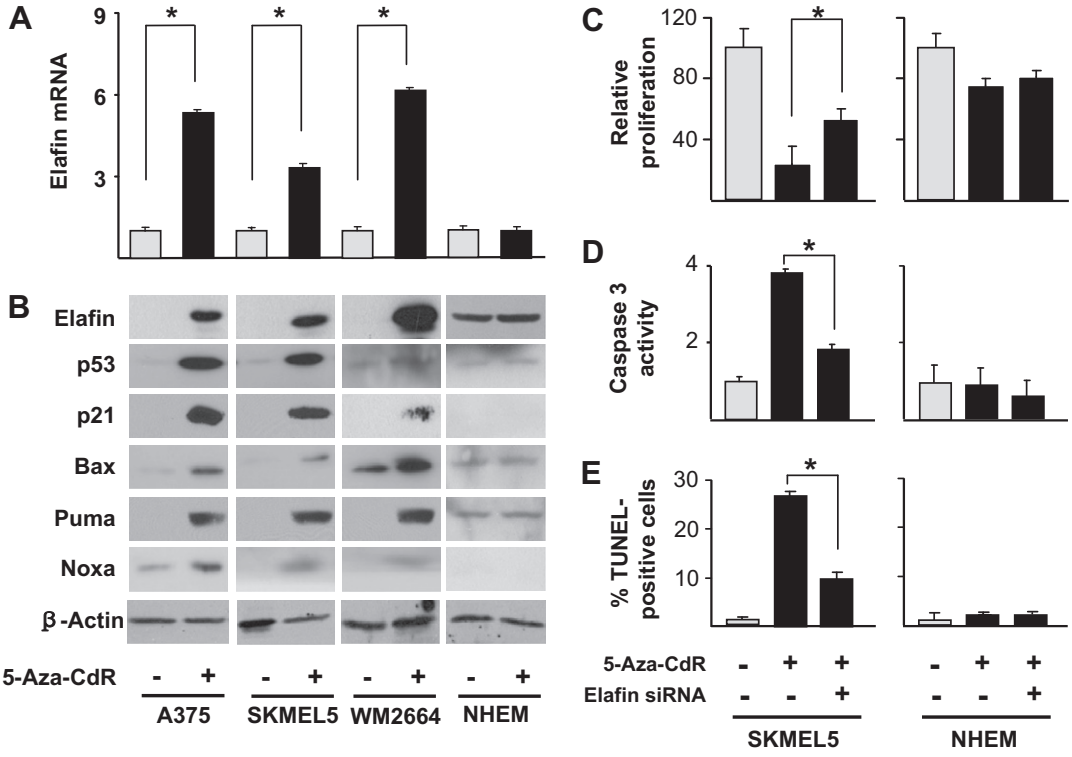

Fig. 1. Elafin is re-expressed in melanoma cells upon treatment with 5-Aza-CdR and induces apoptosis. (A, B) A375, SKMEL5, WM2664, and NHEM cells were treated with 5 μM 5-Aza-CdR (+) or DMSO (−) for 48 h. (A) Total RNA was isolated, and used for quantitative real-time RT-PCR analysis. GAPDH mRNA was used as the normalization control. The elafin mRNA level in DMSO-treated cells was set as 1. (B) Cell lysates were prepared and used for Western analysis. (C–E) SKMEL5 and NHEM cells were transfected with either elafin siRNA (+) or control siRNA (−). After 4 h of transfection, cells were treated with 5 μM 5-Aza-CdR (+) or DMSO (−) for 48 h. (C) Cell numbers were counted and expressed as a percentage of control siRNA- and DMSO-treated cell proliferation. (D) Cell lysates were prepared and assayed for caspase-3 activity. The caspase-3 activities of control siRNA- and DMSO-treated cells were set as 1. (E) TUNEL-positive cells were quantified and presented as a percentage of the total cell count. $^{*}p < 0.05$.

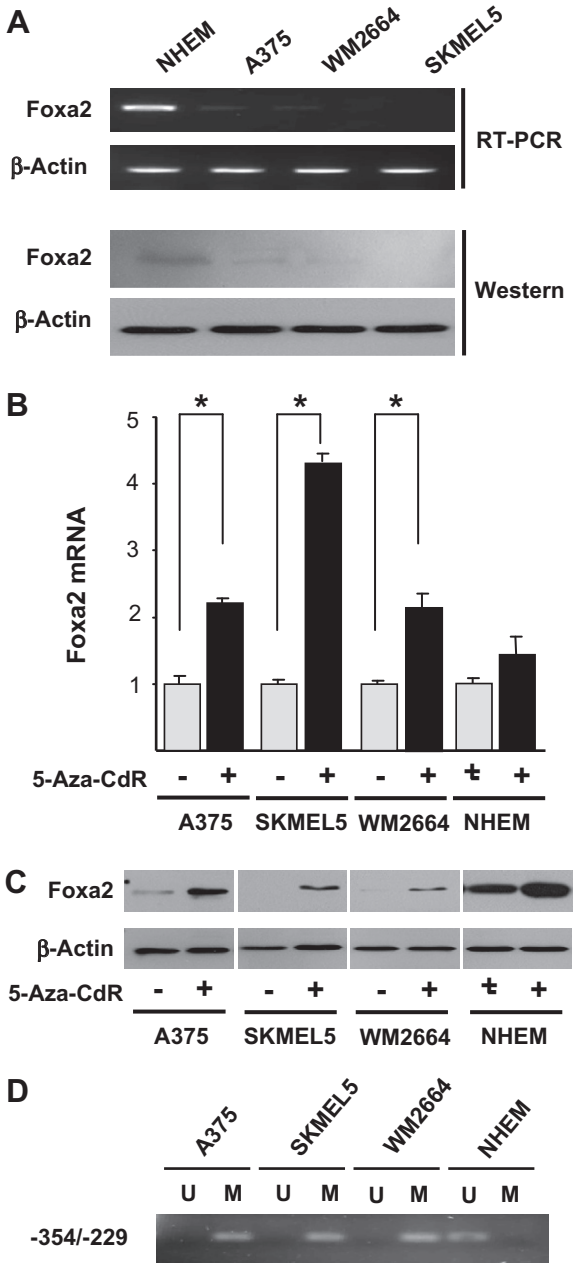

Fig. 2. The Foxa2 promoter is hypermethylated in melanoma cells. (A) Total RNA and cell lysates of NHEM, A375, WM2664, and SKMEL5 were prepared and used for RT-PCR analysis and Western analysis, respectively. (B, C) Cells were treated with 5 μM 5-Aza-CdR (+) or DMSO (−) for 48 h. (B) Total RNA was isolated, and subjected to quantitative real-time RT-PCR analysis. Normalization was performed as for Fig. 1A. The Foxa2 mRNA level in DMSO-treated cells was set as 1. $^{*}p < 0.05$. (C) Cell lysates were prepared and used for Western analysis. (D) Genomic DNA isolated from cells was used for methylation-specific PCR with primers for methylated (M) or unmethylated (U) sequences of the Foxa2 promoter encompassing positions −354 to −229.

Foxa2 mRNA in melanoma cell lines, but only a marginal increase in normal melanocytes (Fig. 2B). Foxa2 mRNA expression in the presence of 5-Aza-CdR was well correlated with the corresponding protein level, as assessed using Western blot analysis (Fig. 2C). The methylation status of the Foxa2 gene was determined in melanoma cells and melanocytes using methylation-specific PCR (MSP). MSP primers were designed to encompass positions −354 to −229 of the Foxa2 promoter containing a CpG island, predicted by CpG island prediction software programs. The MSP results indicated that the Foxa2 promoter is methylated in melanoma cells, but rarely methylated in melanocytes (Fig. 2D). These results demonstrate epigenetic regulation of the Foxa2 gene by DNA hypermethylation in melanoma cells, but not melanocytes.

3.3. Foxa2 directs activation of the elafin promoter in vivo

We reasoned that elafin expression is indirectly triggered by 5-Aza-CdR through reactivation of Foxa2 expression in melanoma cells. To test this possibility, we constructed the reporter plasmid pGR330 in which expression of the firefly luciferase gene was driven by the 700 bp upstream of the elafin promoter bearing the Foxa2 binding site (Fig. 3A). The elafin promoter activity of pGR330 was ~3- to 10-fold lower in melanoma cells compared to that in normal melanocytes (Fig. 3B). The pGR330 promoter, but not the pGR331 promoter containing a mutant Foxa2 binding site, was activated by 5-Aza-CdR in melanoma cells (Fig. 3C). We next determined whether the transcription factor C/EBPβ is involved in elafin transcription in melanoma cells, since C/EBPβ has been shown to activate elafin transcription in breast cancer cells by direct binding to its consensus sequences in the promoter region [11]. Melanoma cells transfected with the reporter plasmid pGR332 harboring mutations in the C/EBPβ binding sites of the elafin promoter still exhibited significant, albeit somewhat reduced, elafin promoter activity upon treatment with 5-Aza-CdR (Fig. 3C), indicating a minor role of C/EBPβ in elafin transcription in melanoma cells. The elafin promoter containing mutations at both Foxa2 and C/EBPβ binding sites (pGR333) displayed little transcriptional activity. The activation patterns of the reporter plasmids in melanoma cells transiently transfected with the Foxa2-expressing plasmid pcDNA-Foxa2 were similar to those in cells treated with 5-Aza-CdR (Fig. 3D). Interactions between Foxa2 and the elafin promoter were further analyzed using the chromatin immunoprecipitation assay. We observed specific binding of Foxa2 to the endogenous elafin promoter in melanoma cells treated with 5-Aza-CdR, whereas binding was limited in untreated cells (Fig. 3E). Similarly, ectopic expression of Foxa2 in melanoma cells resulted in precipitation of the elafin promoter with the anti-Foxa2 antibody (Fig. 3F). These results collectively suggest that Foxa2 is mainly responsible for transcriptional activation of elafin in human melanoma cells by directly binding to its consensus sequence in the elafin promoter.

3.4. Foxa2 expression induces apoptosis of melanoma cells

We reasoned that, in melanoma cells, Foxa2 expression should trigger apoptosis since Foxa2 is a key activator for the expression of elafin which induces the intrinsic apoptotic pathway. Indeed ectopic overexpression of Foxa2 resulted in increased elfin level, and consequently p53, and cleaved caspase 3, which were diminished in the presence of Foxa2-specific siRNA (Fig. 4A). Similar results were obtained from experiments with A375 melanoma cells (data not shown). We observed a significant decrease in proliferation (Fig. 4B) and increase in the TUNEL-positive cell fraction (Fig. 4C) of melanoma cells upon Foxa2 overexpression, compared with the mock control. On the other hand, Foxa2 siRNA attenuated these effects on cell proliferation and apoptosis (Fig. 4B, C). Our results clearly demonstrate the regulated expression of elafin by Foxa2 and the ability of Foxa2 to induce apoptosis in melanoma cells.

4. Discussion

We provide here lines of evidence indicating that silencing of elafin expression in melanoma cells occurs as a result of methylation-mediated suppression of the transcription factor Foxa2. Our findings support a role of Foxa2 as a tumor suppressor in mela-

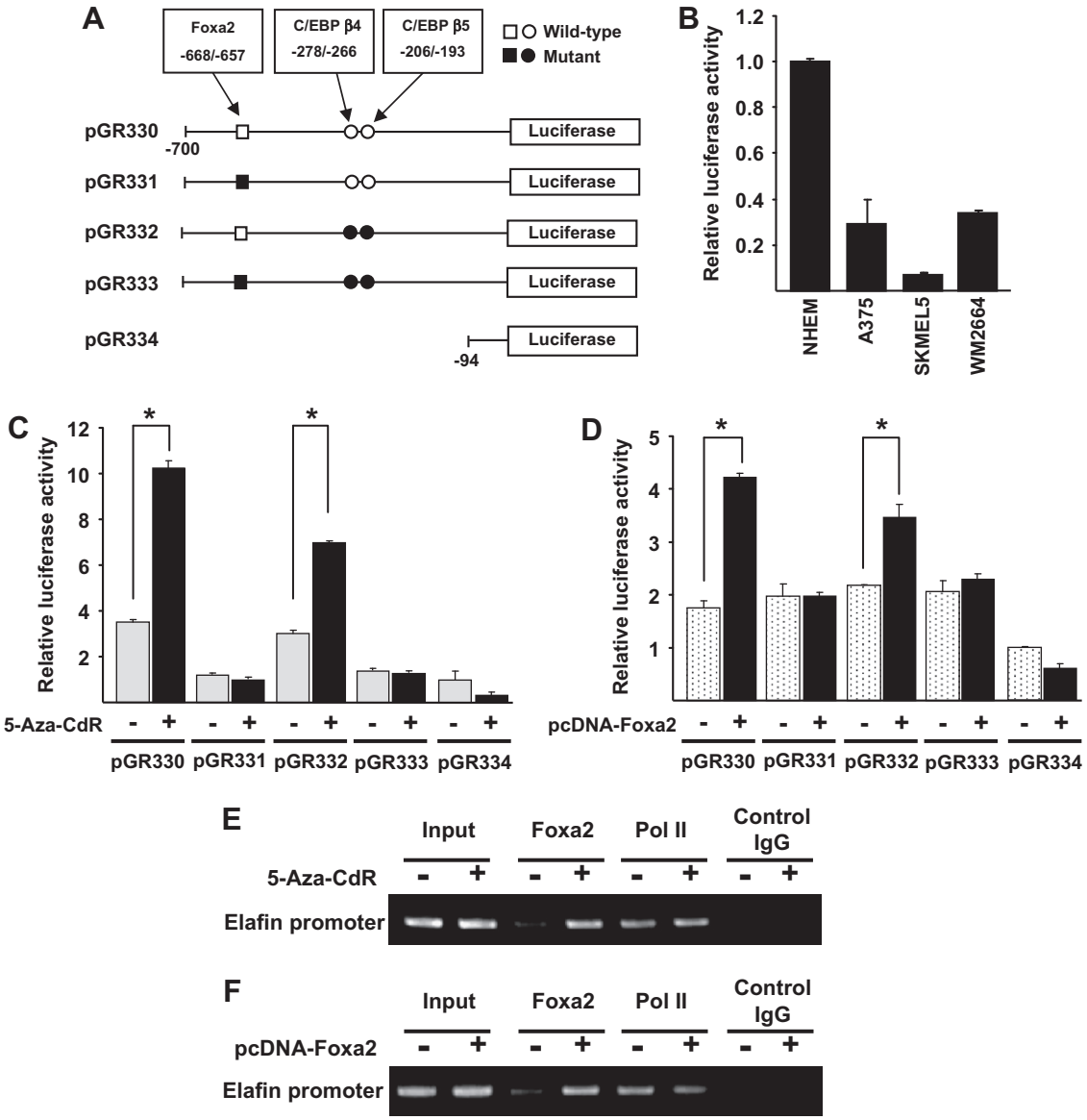

Fig. 3. Foxa2 directs transactivation of the elafin promoter in melanoma cells. (A) Schematic representation of the elafin promoter–luciferase reporter plasmids. Numbering is relative to the first base of the ATG translation codon of the elafin gene. □, wild-type Foxa2 binding site; ■, mutant Foxa2 binding site; ○, wild-type C/EBPβ binding site; ●, mutant C/EBPβ binding site. (B) NHEM, A375, WM2664, and SKMEL5 cells were transfected with pGR330, together with pRL-TK, and analyzed for luciferase activity. The value of the normalized luciferase activity of NHEM was set as 1. (C) A375 cells were transfected with individual reporter plasmids plus pRL-TK. After 24 h, cells were treated with 5 μM 5-Aza-CdR (+) or DMSO (−) for 48 h, and analyzed for luciferase activity. The value of the normalized luciferase activity of pGR344-transfected, DMSO-treated A375 was set as 1. (D) A375 cells were transfected with individual reporter plasmids plus pRL-TK, along with pcDNA-Foxa2 (+) or pcDNA3.1 (−), and analyzed for luciferase activity. The value of the normalized luciferase activity of pGR344- and pcDNA3.1-transfected A375 was set as 1. $^{*}p < 0.05$. (E) Chromatin immunoprecipitation experiments were performed using A375 cells treated with 5 μM 5-Aza-CdR (+) or DMSO (−) for 48 h. Immunoprecipitations were with anti-Foxa2, anti-RNA polymerase II (positive control), and control mouse IgG (negative control). (F) Chromatin immunoprecipitation experiments were performed using A375 cells transiently transfected with pcDNA-Foxa2 (+) or pcDNA3.1 (−) for 48 h.

noma, consistent with previous reports on Foxa2 functions in other cancer types. In thyroid cancer cells, Foxa2 expression is silenced due to promoter methylation, and forced expression of Foxa2 leads to suppression of cell growth [18]. Conditional expression of Foxa2 in lung cancer cells, where the factor is downregulated by methylation, leads to growth reduction, proliferation arrest, apoptosis, and loss of clonogenic ability [19]. In pancreatic ductal adenocarcinoma samples, Foxa2 is constitutively expressed in normal epithelial and well-differentiated cancer cells, but suppressed in undifferentiated cancer cells by methylation-dependent regulation [17]. Ectopic Foxa2 expression reactivates E-cadherin expression, leading to inhibition of epithelial-to-mesenchymal transition of pancreatic cancer cells. Similarly, Foxa2 is not expressed due to promoter methylation in breast cancer cell lines but is expressed in normal mammary epithelial cells [20], and metastatic breast cancer cells stably transfected with Foxa2-expressing plasmid exhibit induced E-cadherin expression, mesenchymal-to-epithelial transition, and reduced cell motility [22]. In our experimental settings, Foxa2 expression in melanoma cells did not induce E-cadherin expression or mesenchymal-to-epithelial transition but led to decreased cell proliferation and apoptosis through reactivation of elafin transcription, suggesting differential downstream target genes of the transcription factor depending on cancer types.

Temporal and spatial regulation of Foxa2 by other transcription factors may account for its differential functions in distinct tissue environments. A recent report shows that a limited subset of the hypoxia inducible factor-1α (HIF-1α) target genes is specifically induced by cooperation between HIF-1α and Foxa2, promoting the

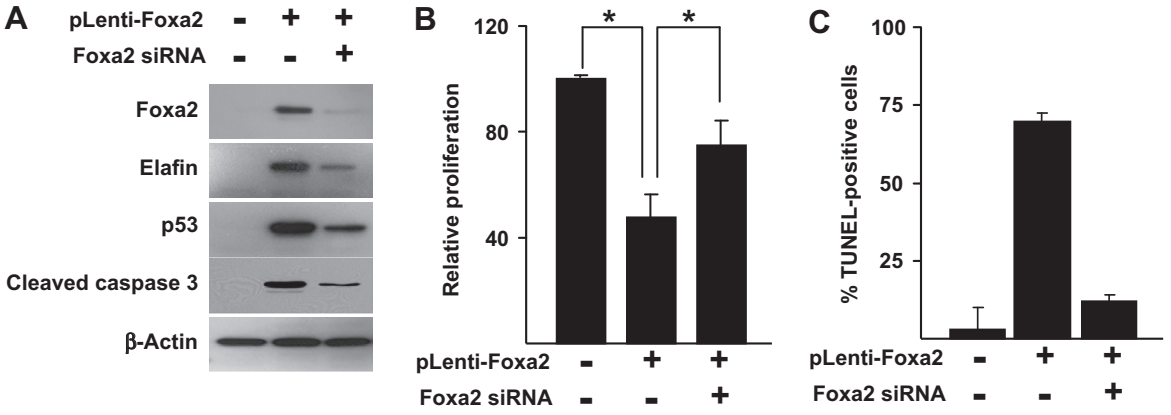

Fig. 4. Foxa2 induces apoptosis of melanoma cells. (A) Lentivirus bearing pLenti-Foxa2 (+) or pLentiM1.3hygro (−) was introduced into SKMEL5 cells, along with Foxa2 siRNA (+) or control siRNA (−). After 48 h, cell lysates were prepared and used for Western analysis. (B) Cell numbers were counted, and expressed as a percentage of pLentiM1.3hygro- and control siRNA-treated control cell proliferation. $^{*}p < 0.05$. (C) TUNEL-positive cells were quantified and expressed as a percentage of the total cell count.

development of a hypoxia-dependent neuroendocrine (NE) phenotype in prostate tumors [23,24]. Foxa2 is expressed in mouse prostate NE carcinomas [25] and NE foci of human prostate adenocarcinoma [26]. As another example, unacetylated hepatocyte nuclear factor 6 (HNF6) is highly expressed in colorectal liver metastasis, but fails to activate its downstream target genes due to interactions with Foxa2, which is also induced [27]. A model in which Foxa2 and HNF6 interactions either synergistically stimulate or repress transcription, dependent on target sequences, was previously suggested by Rausa et al. [28]. Elafin expression involves transcription factors such as NF-κB in ovarian cancer cells [5], and C/EBPβ [11] and Ap1 [13] in breast cancer cells. Our data demonstrate Foxa2 to have a major role in elafin expression in melanoma cells. To date, no studies have reported interactions between Foxa2 and C/EBPβ, NF-κB, or Ap1. Identification of Foxa2-interacting transcription factors and determination of communications, either positive or negative, in the context of melanoma should provide us detailed regulatory mechanism underlying elafin transactivation.

We suggest that epigenetic repression of Foxa2, and thus silencing of elafin expression, in melanoma cells might be necessary for progression and/or maintenance of the disease. Comprehensive histological studies with patient tissue specimens may further reveal the clinical implications of Foxa2 silencing, and provide a rationale for therapeutic interventions to treat melanoma.

Conflict of interest

All the authors declare no conflict of interest.

Acknowledgments

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