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# Slug Regulates E-cadherin Repression via p19Arf in Prostate Tumorigenesis

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## Abstract

SLUG represses E-Cadherin to promote epithelial-mesenchymal transition (EMT) in various cancers. Mechanisms that regulate SLUG/E-Cadherin pathway remain poorly understood, especially during tumorigenesis *in vivo*. Here we report that $p19^{Arf}$ ($p14^{ARF}$ in human) stabilizes Slug to inhibit E-Cadherin in prostate cancer mouse models. Inactivation of $p19^{Arf}$ reduces Slug levels, resulting in increased E-Cadherin expression and delaying the onset and progression of prostate cancer in *Pten/Trp53* double null mice. Mechanistically, $p14^{ARF}$ stabilizes SLUG through increased sumoylation at lysine residue 192. Importantly, levels of SLUG and $p14^{ARF}$ are positively correlated in human prostate cancer specimens. These data demonstrated that ARF modulates the SLUG/ECadherin signaling axis for augmenting prostate tumorigenesis *in vivo*, revealing a novel paradigm where the oncogenic functions of SLUG require ARF to target E-Cadherin in prostate cancer. Collectively, our findings further support that ARF has dual tumor suppressive/oncogenic roles in cancers in a context-dependent manner.

### Keywords

SLUG; prostate cancer; SUMO1; mouse model



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## 1. Introduction

Prostate cancer (PCa) is driven by aberrant regulations of multiple pathways involving in many tumor suppressors and oncogenes (Thomas et al., 2008). PTEN (phosphatase and tensin homolog deleted on chromosome 10) is frequently mutated and deleted in human cancers including PCa (Whang et al., 1998). Inactivation of PTEN contributes to hyper-activation of the AKT-mTOR (mammalian target of rapamycin) signaling pathway and downstream targets (Cantley and Neel, 1999). Complete loss of Pten triggers a cellular senescence response through the aberrant activation of the ARF-p53 pathway (Chen et al., 2005; Yilmaz et al., 2006), but eventually results in invasive PCa in mice (Pourmand et al., 2007). Inactivation of this senescence pathway through loss of *Trp53* augments cell proliferation, leading to a lethal cancer phenotype (Chen et al., 2005; Zheng et al., 2008). By contrast, inactivation of $p19^{Arf}$ reduces, instead of accelerates, prostate tumorigenesis driven by *Pten*-deficiency, indicating the oncogenic potential of ARF in the prostate. Yet, the mechanism contributed by ARF remains elusive.

ARF ($p14^{ARF}$ in human or $p19^{Arf}$ in mouse) is an alternative transcript of the *ARFINK4a* locus (*CDKN2a*) on human chromosome 9q21 that encodes two cyclin-dependent kinase inhibitors, $p14^{ARF}$ and $p16^{INK4a}$ (den Besten et al., 2006; Kamijo et al., 1999). *CDKN2a* deficiency causes spontaneous or increased susceptibility to carcinogen-induced tumors (Bardeesy et al., 2006; Sharpless et al., 2004). ARF is functionally coupled to p53 through antagonization of the Mdm2-mediated p53 degradation, and overexpression of $p19^{Arf}$ results in a p53-dependent growth arrest in fibroblasts (Chen et al., 2010; Ha et al., 2007). Increasing evidence reveals that ARF may function in both tumor suppressive and oncogenic roles in cancers depending on the context (Abida and Gu, 2008; Humbey et al., 2008; Khoo et al., 2007). For example, $p19^{Arf}$-deficient mice develop sarcomas, lymphomas, pulmonary and mammary adenocarcinomas; but the morphology of prostate remains normal (Kamijo et al., 1999). ARF protein is dramatically accumulated in cells upon oncogenic insults such as loss of Pten, or activation of Ras or Myc (Lowe and Sherr, 2003). We have previously shown that increased ARF expression is correlated with aggressive disease in human PCa specimens (Chen et al., 2009), and recent studies reveal that ARF absence causes apoptosis in spermatogonia of mice (Churchman et al., 2011). These results suggest that ARF may execute a unique oncogenic function or may be required for sustained cell proliferation under specific contexts.

SLUG promotes cell migration and cancer metastasis through repression of ECadherin (Hajra et al., 2002) and the regulation of epithelial-mesenchymal transition (EMT) (Shih and Yang, 2011). Even though, the underlying mechanisms regulating SLUG during PCa progression are still poorly understood (Rodriguez et al., 2012), particularly in the context of Pten loss. Sumoylation is mediated via SUMO1 or SUMO2/3 to regulate kinase activity as well as protein trafficking and stability (GeissFriedlander and Melchior, 2007; Kho et al., 2011), and ARF is involved in protein post-translational regulation including sumoylation (den Besten et al., 2006; Tago et al., 2005). We hypothesized that the oncogenic function of ARF in PCa may be associated with its role in the post-translational regulation of SLUG. Here we define a novel mechanism in that ARF enhances SLUG sumoylation resulting in the repression of ECadherin during PCa progression.

## 3. Results

### 3.1. *p19$^{Arf}$* deficiency suppresses prostate tumor progression in *Pten/Trp53* double-null mice

We have previously shown that *Pten/Trp53* mutant mice develop aggressive PCa, and increased ARF expression is observed in this mouse model as well as PCa specimens with malignant phenotypes. To understand the role of p19$^{Arf}$ in prostate tumorigenesis, we generated prostate specific *Pten/Trp53/p19$^{Arf}$* triple knockout mice. As *Pten/Trp53* mutant mice developed PCa after puberty, prostate tissues of mutant mice from two groups were collected at 3 months of age, and were subjected to histopathological analysis. Interestingly, the mass of the anterior prostate lobes (AP) in *Pten/Trp53/p19$^{Arf}$* mutant mice (both *p19$^{Arf+/-}$* and *p19$^{Arf-/-}$*) were reduced by approximately 50% compared to that of age-matched *Pten/Trp53* mice, though *Pten/Trp53/p19$^{Arf}$* mice exhibited enlarged prostates (Supplementary Figure S1A and B, n=12, $p$=0.059). Pathological analysis revealed that *Pten/Trp53/p19$^{Arf}$* mice developed high-grade prostatic intraepithelial neoplasia (HG-PIN) but not invasive cancer in all three prostate lobes, compared to *Pten/Trp53* mice that displayed a significantly increased volume of HG-PIN and invasive cancer (Supplementary Figure S1C). These results indicated that deletion of p19$^{Arf}$ resulted in a marginal suppression on the initiation of prostate tumorigenesis of *Pten/Trp53* mice at 3 months of age. In contrast, the growth of prostate tumors of *Pten/Trp53/p19$^{Arf}$* mice was remarkably suppressed as compared to that of *Pten/Trp53* mice at 6 months of age (Figure 1A). The average tumor weight of *Pten/Trp53/p19$^{Arf}$* mice was reduced 3.7-fold as compared to that of *Pten/Trp53* mice (Figure 1B; n=12, $p$<0.001, *t*-test). To evaluate the sustained impact of p19$^{Arf}$ in tumors of *Pten/Trp53* mice, we followed a cohort of 52 mice over 9 months for cumulative survival analysis. Remarkably, the reduction of p19$^{Arf}$ prolonged the overall survival rate of *Pten/Trp53* mice (Figure 1C), and loss of one allele of *p19$^{Arf}$* showed a suppressive effect on tumor growth (Figure 1A-C). Therefore, p19$^{Arf}$ inactivation significantly suppresses the PCa progression in *Pten/Trp53* mice.

### 3.2. p19$^{Arf}$ inactivation represses the EMT phenotype of *Pten/Trp53* mice through regulation of SLUG

Next we performed molecular pathological analyses on the prostate tumors of *Pten/Trp53* and *Pten/Trp53/p19$^{Arf}$* mice at 6 months of age. Our results showed that prostate tumors of *Pten/Trp53/p19$^{Arf}$* mice maintain differentiated proliferations of prostate epithelial cells, and *Pten/Trp53* mice develop de-differentiated histologies displaying an EMT-like phenotype with the transition from epithelial to mesenchymal (sarcomatoid) (Figure 2A, Supplementary Figure S2A). Importantly, IHC staining showed that Slug, a marker of EMT, was strikingly elevated in tumors of *Pten/Trp53* mice, and p19$^{Arf}$ inactivation significantly decreased Slug levels (Figure 2B). As SLUG targets the down-regulation of E-Cadherin in cancers (Hajra et al., 2002), we examined E-cadherin levels in the tumors. As expected, E-Cadherin levels were markedly reduced in *Pten/Trp53* mice where levels of p19$^{Arf}$ and Slug were elevated, but were not increased in *Pten/Trp53/p19$^{Arf}$* mice where *p19$^{Arf}$* is deleted and Slug levels were low (Figure 2C, Supplementary Figure S2B). Prostatic cells of *Pten/Trp53/p19$^{Arf}$* mice showed decreased proliferations when compared to *Pten/Trp53* mice, as

evidenced by Ki67 staining (Figure 2D). Thus our results suggest that $p19^{Arf}$ deficiency restricts tumorigenesis through regulation of Slug and E-Cadherin.

### 3.3. ARF determines SLUG protein level in human prostate cancer cells

To investigate the regulation of SLUG by ARF, we knocked down $p14^{ARF}$ in PC3 cells that have high levels of ARF and SLUG (Data not shown). Our results showed that $p14^{ARF}$ knockdown resulted in a striking reduction of SLUG protein levels as compared to the control, and the down-stream expression of E-Cadherin was increased while Vimentin levels were decreased (Figure 3A). Furthermore, $p14^{ARF}$ knockdown resulted in a significant reduction of cell proliferation (Figure 3B), wound closure, and cell migration (Figure 3C and D), and a tightly adhesive morphology of epithelial cells when compared to control cells (Supplementary Figure S3). In agreement with previous reports (Chen et al., 2009; Churchman et al., 2011), our results reveal that $p14^{ARF}$ regulates SLUG protein levels thereby promoting cell proliferation and migration in PCa.

### 3.4. ARF regulates SLUG levels through induction of sumoylation in prostate cancer cells

To understand the mechanism of ARF-mediated SLUG regulation, we examined whether ARF regulates SLUG gene transcription. Our results showed that $p14^{ARF}$ overexpression did not result in the transactivation of luciferase reporter gene driven by the SLUG-promoter (Figure 4A), indicating SLUG regulation by $p14^{ARF}$ may not occur at the transcriptional level. We reasoned that SLUG may be regulated by ARF in a post-transcriptional fashion. As demonstrated, $p14^{ARF}$ knockdown resulted in a remarkable reduction of the half-life of SLUG protein levels as compared to the control (Figure 4B), suggesting that ARF is essential in stabilizing the SLUG protein. Given the role of ubiquitination on protein degradation, we tested if MG-132, a proteasome inhibitor, would prevent the observed decrease in SLUG protein levels caused by ARF knockdown. Surprisingly, MG-132 treatment failed to reverse the impact of ARF knockdown (Supplementary Figure S4), suggesting that ARF may stabilize SLUG through a mechanism independent of ubiquitin-mediated proteasomal degradation.

Since ARF plays a role on protein sumoylation (den Besten et al., 2006), we investigated whether ARF stabilizes SLUG through sumoylation. We first determined whether ARF associates with SLUG protein in its sumoylated form. Our results demonstrated that SLUG was co-localized with both ARF and SUMO1 in nucleus of PC3 cells (Figure 4C and D), and SLUG interacted with ARF and SUMO1 (Figure 4E). To further confirm the SLUG sumoylation, cell lysates were subjected to the detection of SLUG modifications in Western blot by three different antibodies. As shown, in addition to the SLUG bands, higher molecular weight bands of SLUG (SLUG-H) were detected by all three antibodies in PC3 cells but not in LNCaP cells in which SLUG levels are very low (Sun et al., 2012) (Figure 4F). Moreover, SLUG-H expression was correlated with ARF elevation and a decrease in E-Cadherin (Figure 4F, left panel), and endogenous SLUG-H was conjugated with SUMO1 (Figure 4F, right panel). Our results indicate that endogenous SLUG is sumoylated in PC3 cells, and ARF interacts with SLUG to promote its sumoylation.

### 3.5. ARF-promoted SLUG sumoylation is essential for protein stability

To evaluate the effects of SLUG sumoylation on protein stability, we co-transfected Myc-SLUG with SUMO1, SUMO1 glycine-deleted mutant (ΔGG), or SUMO2/3 into 293FT cells followed by co-immunoprecipitation (co-IP). We found that SUMO1 interacted with SLUG, and SUMO1 mutation at ΔGG abolished the interaction, while SUMO2/3 had no interaction with SLUG (Figure 5A). We determined the lysine residues of SLUG that are for sumoylated modifications. The SUMOplot™ Analysis Program (Abgent) predicted that K192 and K258 were two candidate sites of SLUG sumoylation (Supplementary Figure S5A). We then generated K192R and K258R mutants of SLUG, and found that protein levels of the $SLUG^{K192R}$ mutant were remarkably decreased as compared to that of $SLUG^{wt}$ and $SLUG^{K258R}$ (Figure 5B). In addition, the $SLUG^{K192R}$ mutant maintained a similar pattern of cellular localization to that of $SLUG^{wt}$ (Figure 5C). These results suggested that K192 could be the major site of SLUG sumoylation in regulating SLUG protein levels. To examine the effect of K192R mutation on SLUG stability, we performed CHX chase experiments and found that the K192R mutation decreased the half-life of SLUG protein (Figure 5D), suggesting that SLUG sumoylation at K192 is essential for its stability. Consistent with the sumoylated SLUG normally seen as a higher band in Western blotting, the K192R mutation showed a decreased level of the high molecular weight form of SLUG (SLUG-H) (Supplementary Figure S5B). $SLUG^{K192R}$ mutation reduced its interaction with SUMO1 compared to $SLUG^{wt}$ (Figure 5E). Our results suggest that the SLUG sumoylation predominately occurs at K192 by SUMO1 to increase protein stability.

As SLUG sumoylation is required for its stability, we asked if ARF stabilized the SLUG protein through increasing the interaction between SLUG and SUMO1 for sumoylation. The co-IP assay revealed that ARF overexpression resulted in a 2-fold increase of SUMO1 binding with SLUG as compared to the control (Figure 5F). Consistent with the results in PC3 cells, overexpression of SLUG also showed an association with ARF and SUMO1 (Figure 5F). Similarly, ARF knockdown by two individual shRNA constructs dramatically decreased SLUG and SLUG-H (Data not shown). Together, our results demonstrated that ARF promotes SLUG sumoylation to increase the protein stability of SLUG.

### 3.6. SLUG protein levels correlate with ARF expression in human prostate cancer specimens

The novel function of ARF on SLUG *in vitro* and *in vivo* encouraged us to assess the correlation of their protein levels in human PCa specimens. IHC staining of prostate tissue microarrays revealed that ARF and SLUG protein levels were significantly higher in advanced stages of PCa (IV) compared to normal tissues (Figure 6A). Our results are in agreement with previous reports that either ARF or SLUG levels alone are significantly elevated in advanced cancers (Chen et al., 2009; Liu et al., 2012). Importantly, we found that the correlation between ARF and SLUG protein levels were statistically significant in PCa specimens (Figure 6B, $r= 0.6891$, $p < 0.01$). PCa samples with elevated SLUG displayed higher levels of ARF (Figure 6C). Thus, ARF may be an important factor in maintaining increased levels of SLUG during PCa progression.

## 4. Discussion

ARF was recently reported to show pro-proliferative or oncogenic-promoting roles in cells under compromised physiologic or genetic contexts (Chen et al., 2009; Humbey et al., 2008; Mulholland et al., 2012). In this study, we demonstrated *in vitro* and *in vivo* that ARF contributes to PCa progression by regulation of the SLUG and E-Cadherin axis. Furthermore, we identified mechanistically that ARF regulates the post-translational modification of SLUG.

Loss of E-Cadherin, a hallmark of cancer, is primarily controlled by elevation of SNAIL and SLUG proteins (Cano et al., 2000). Deletions and mutations of *PTEN* and *p53* are frequently found in various cancers including advanced PCa (Shen and Abate-Shen, 2010), and inactivation of both PTEN and p53 in the prostate leads to the lethal cancer in mice. Loss of PTEN can induce an EMT to accelerate PCa progression in the RAS-activated mouse model (Dubrovska et al., 2009; Mulholland et al., 2012). Additionally, p53 deficiency promotes an EMT in breast cancer cells through miR-200c (Chang et al., 2011). It has been observed that loss of PTEN and p53 promotes an EMT in PCa, yet the mechanism was not well understood. Our results reveal that the EMT-like phenotype induced by PTEN and p53 loss is an ARF-dependent oncogenic cascade that induces SLUG sumoylation to increase the protein stability resulting in down-regulation of E-Cadherin. This mechanism underscores the oncogenic role of ARF in cell motility and malignancy in the prostate, which is different from that of other tissue contexts where $p19^{Arf}$ is reported to inhibit cell motility by the suppression of Pten/PI3K deregulation or Rac1 GTPase (Guo et al., 2003; Yu et al., 2009). Our study reveals a novel network among PTEN/PI3K/AKT, ARF, and EMT signaling in PCa.

One noteworthy finding in this study is that ARF stabilizes SLUG protein to potentiate its oncogenic functions in PCa cells. Given the essential role of the SLUG/E-Cadherin axis in cancer progression, our discovery that ARF modulates the protein interactions between SUMO1 and SLUG for SLUG sumoylation resulting in decreased E-Cadherin provides valuable insight into understanding the role of sumoylation in malignancy. Our studies have defined that the aberrant elevation of ARF upon PTEN and p53 inactivation determines the oncogenic switch from p53-dependent senescence to SLUG-driven EMT (Kuilman et al., 2010) (Figure 6D). Collectively, our findings highlight that ARF-mediated stabilization of SLUG is important for the repression of cell-cell junctions and E-Cadherin during cancer progression (Figure 6D). Further investigation will reveal how ARF overexpression cooperates with SLUG signaling to regulate cancer cell invasion and metastasis, and the impact of SLUG sumoylation deficiency in PCa *in vivo*. We previously reported that ARF inhibits androgen receptor (AR) activity by direct disruption of NH and COOH terminal interactions of AR (Lu et al., 2013). AR is reported to regulate an EMT by targeting E-Cadherin (Liu et al., 2008). Here we demonstrated that ARF regulates SLUG and E-Cadherin both in AR positive (data not shown) and AR negative PCa cells, indicating that ARF can stabilize SLUG to repress E-Cadherin in both an AR dependent and independent manner (Sun et al., 2012). This suggests that ARF plays distinct roles in mediating AR action and EMTs.

We previously reported that ARF correlates with Gleason scores in human prostate cancer specimens (Chen et al., 2009). Here we showed ARF correlates with SLUG, an EMT marker, in advanced stages of PCa, highlighting that SLUG and ARF have the potential of being biomarkers for aggressive PCa. Our findings on the association of SLUG sumoylation with EMT constitute important clues with implications in clinical application. Chemotherapeutic compounds targeting ubiquitination machinery to control cancers have been in clinical trials. With a similar strategy, inhibitors preventing SLUG sumoylation may constitute a novel strategy for reversal of EMT to thereby block cancer metastasis. Furthermore, due to the oncogenic functions of ARF, treatments targeting both ARF and SLUG sumoylation would be of potential therapeutic strategy to improve the efficacy for PCa control.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Non-standard abbreviations

| | |
|---|---|
| **EMT** | epithelial-mesenchymal transition |
| **PCa** | prostate cancer |
| **PTEN** | phosphatase and tensin homolog deleted on chromosome 10 |
| **SUMO1** | small ubiquitin-like modifier 1 |
| **ARF** | alternative reading frame |

## Highlights

- $p19^{Arf}$ deficiency inhibits prostate tumorigenesis of *Pten/Trp53* mice.
- $p19^{Arf}$ inactivation results in Slug decrease and E-Cadherin increase *in vivo*.
- ARF stabilizes SLUG through SUMO1 interaction.
- SLUG sumoylation at K192 residue is essential for the stability.
- Levels of SLUG and ARF are positively correlated in human prostate cancer specimens.

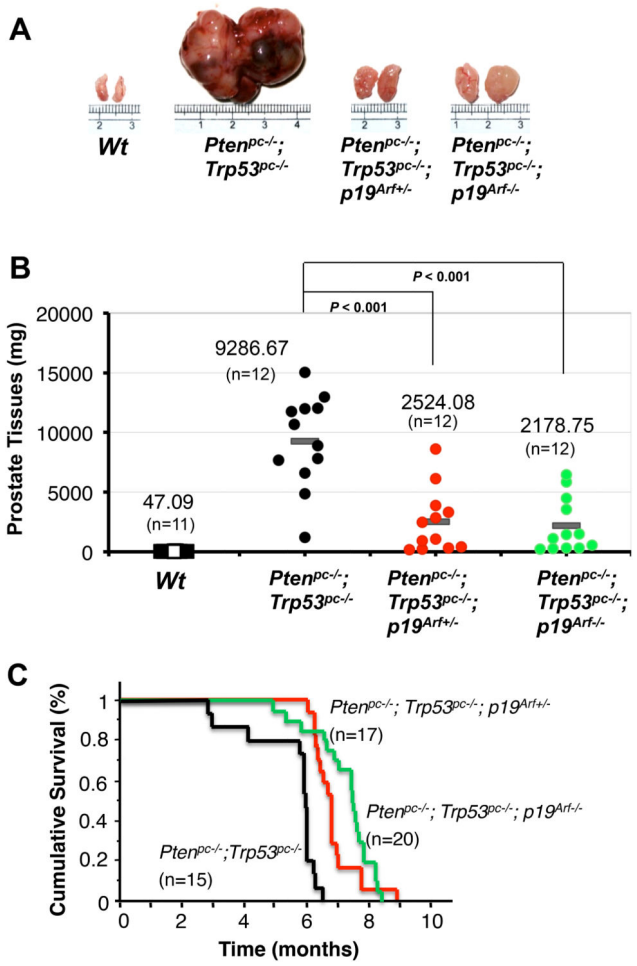


**Figure 1.**

p19$^{Arf}$ deficiency significantly suppresses prostate tumorigenesis in *Pten/Trp53* mice. (A) Actual sizes of representative tumors from anterior prostates (AP) of *Wt*, *Pten*$^{pc-/-}$; *Trp53*$^{pc-/-}$ double-mutant, and *Pten*$^{pc-/-}$;Trp53$^{pc-/-}$;*p19*$^{Arf-/-}$ triple-mutant mice at 6 months of age. (B) Comparison of AP tumor masses from *Pten*$^{pc-/-}$;*Trp53*$^{pc-/-}$, *Pten*$^{pc-/-}$;*Trp53*$^{pc-/-}$;*p19*$^{Arf+/-}$ and *Pten*$^{pc-/-}$;*Trp53*$^{pc-/-}$;*p19*$^{Arf-/-}$ mice at 6 months of age. p19$^{Arf}$ deficiency inhibits prostate tumorigenesis in *Pten*/*Trp53* mutant mice (N=12 for each group, $P<0.001$). (C) Cumulative survival analysis (Kaplan-Meier plot) for *Pten*$^{pc-/-}$;*Trp53*$^{pc-/-}$ (black line), *Pten*$^{pc-/-}$;*Trp53*$^{pc-/-}$;*p19*$^{Arf+/-}$ (red line), and *Pten*$^{pc-/-}$;*Trp53*$^{pc-/-}$;*p19*$^{Arf-/-}$ (green line) cohort mice.

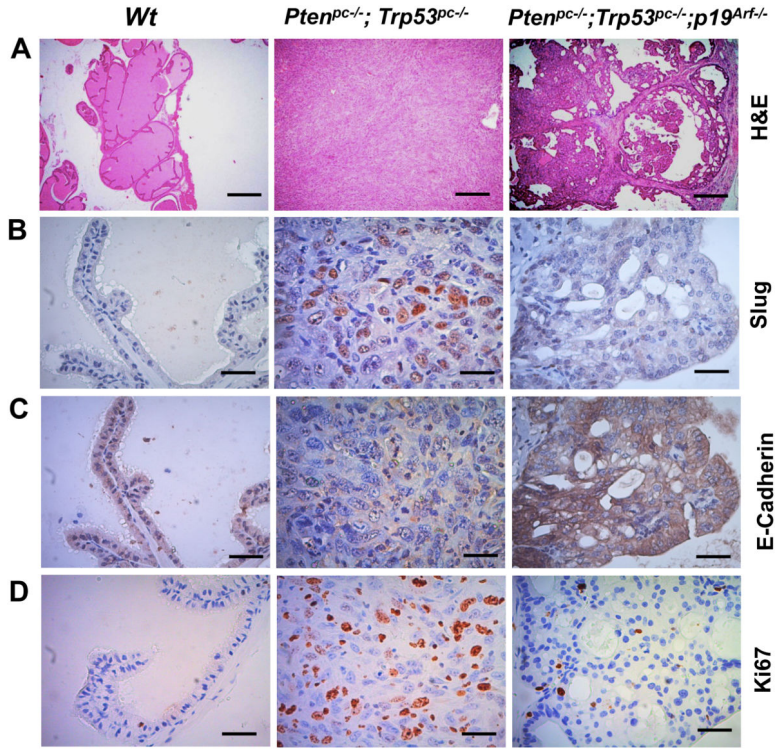


**Figure 2.**

Loss of $p19^{Arf}$ suppresses prostate cancer progression by down-regulating Slug in mice. (A) Histopathology analysis (H&E staining) of anterior prostates in *Wt*, $Pten^{pc-/-}$;$Trp53^{pc-/-}$, $Pten^{pc-/-}$;$Trp53^{pc-/-}$;$p19^{Arf-/-}$ mutant mice at 6 months of age. Immunohistochemical staining (IHC) of Slug (B), E-Cadherin (C) and Ki67 (D) in prostate tissue (A). Bars equal 500μm (A), 50 μm (B,C,D).

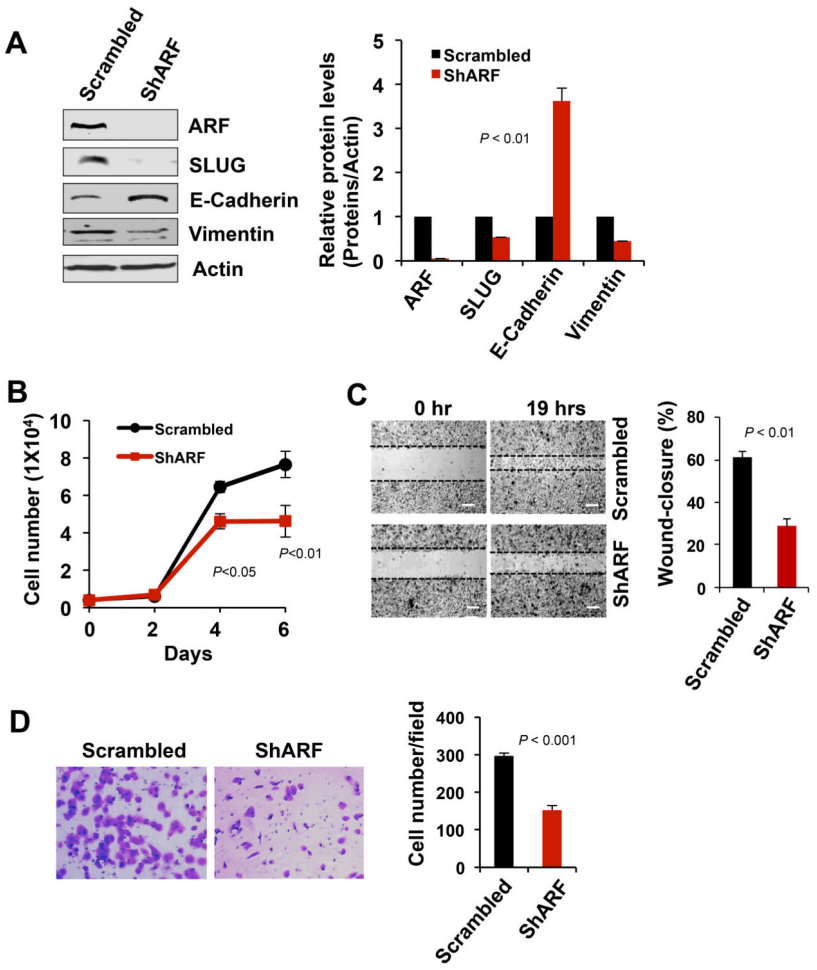


**Figure 3.**

Effects of p14$^{ARF}$ knockdown on SLUG, cell growth and migration in prostate cancer cells. (A) SLUG reduction upon p14$^{ARF}$ knockdown in PC3 cells. E-cadherin is increased and Vimentin is decreased. Quantifications on Western blot results were shown at the right panel. (B) Decreased proliferation of PC3 cells upon p14$^{ARF}$ knockdown. (C) Reduced wound-healing ability of PC3 cells by p14$^{ARF}$ knockdown. Bars equal 100μm. (D) Decreased migration of PC3 cells u pon p14$^{ARF}$ knockdown. Migrated cells were counted from three fields and presented as mean values ± s.d. ** $p$<0.01 indicates the statistical significance, Student's $t$-test, n=3.

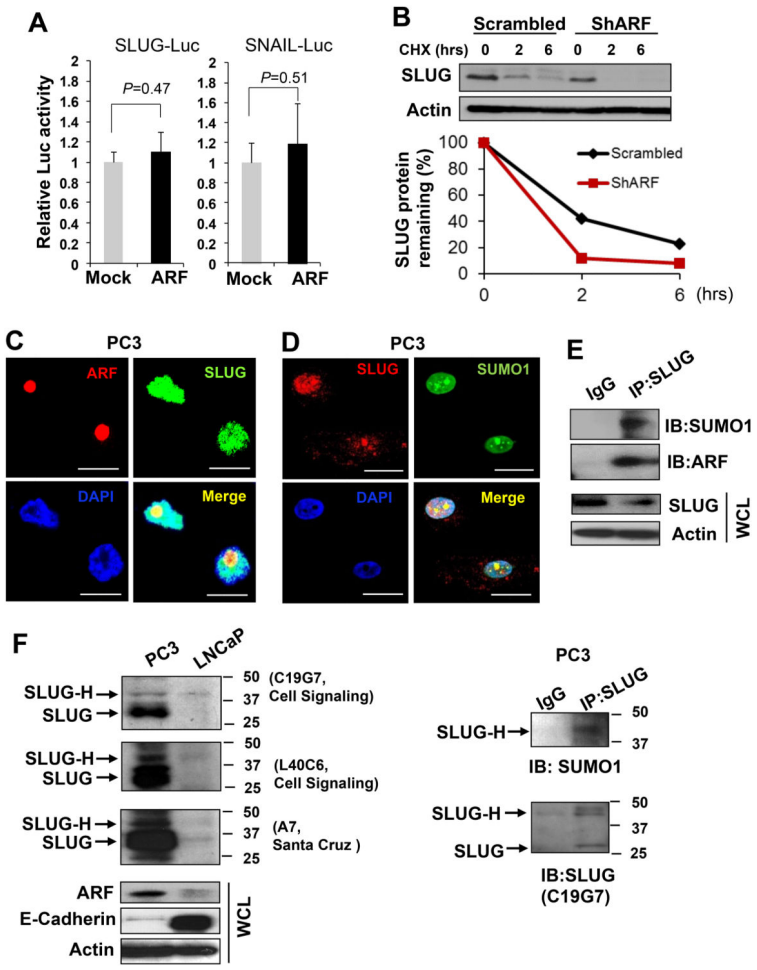


**Figure 4.**

The association of ARF with SLUG determines SLUG stability. (A) ARF overexpression does not affect the promoter activities of *SLUG* and *SNAIL* genes in Luciferase reporter assay. (B) ARF knockdown results in a shortened half-life of SLUG protein in PC3 cells. (C) Co-localization of ARF and SLUG proteins in nucleus of PC3 cells. Bars equal 25μm. (D) Co-localization of SLUG and SUMO1 in PC3 cells. (E) Coimmunoprecipitation (IP) was performed using SLUG (L40C6) antibody, and immunoblot (IB) was probed using ARF (4C6/4) or SLUG (C19G7) antibody. (F) Left panel: detection of endogenous SLUG using three different antibodies to show a higher molecular weight band (SLUG-H) at 42kDa and lower molecular weight band (SLUG) at 30kDa in prostate cancer cells. Right panel: co-IP using SLUG (A7) antibody and IB using SLUG (C19G7) or SUMO1 to show the SLUG-H and SUMO1 band in PC3 cells.

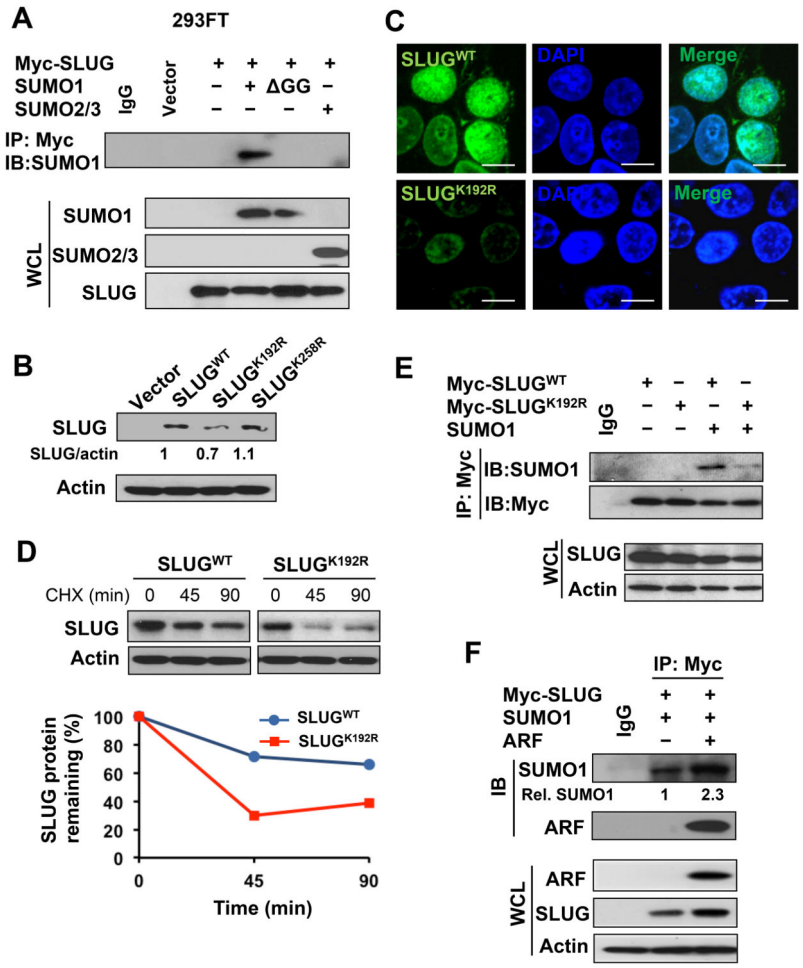


**Figure 5.**

SLUG sumoylation is essential for its protein stability. (A) Interaction between SLUG and SUMO1 for sumoylation. (B) SLUG mutation at K192R decreases SLUG protein levels. (C) Localization of $SLUG^{K192R}$ Pin nucleus of 293FT cells. Myc-tag antibody was used to detect Myc-SLUG. Bars equal 10 μm. (D) $SLUG^{K192R}$ decreases the protein stability compared to $SLUG^{WT}$. (E) $SLUG^{K192R}$ decreases the interaction with SUMO1 compared to $SLUG^{WT}$. (F) ARF promotes the interaction between SUMO1 and SLUG for sumoylation.

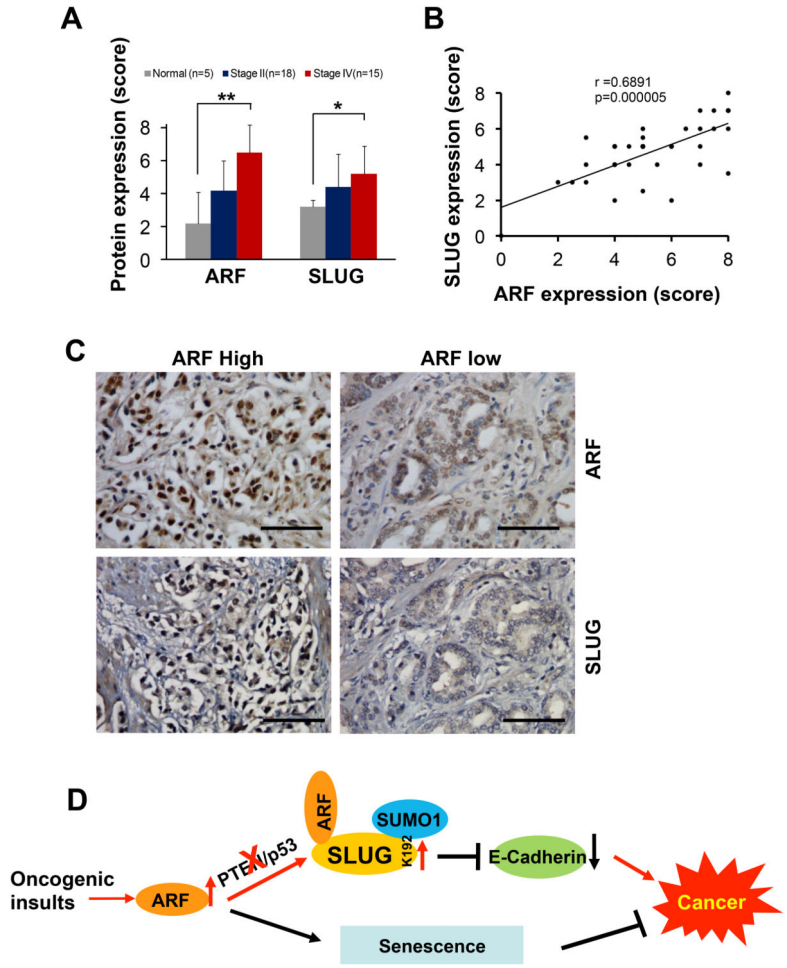


**Figure 6.**

The correlation between ARF and SLUG in human prostate cancer. (A) Levels of ARF and SLUG expression among normal, stages II and IV of prostate cancer. SUMO1 compared to SLUG CWT **, $p<0.01$ and *, $p<0.05$ indicate the statistical significance by Student's *t*-test. (B) Statistical analysis of the correlations between ARF and SLUG, in human prostate cancer tissue specimens. (C) IHC staining of ARF and SLUG in human prostate cancer tissues. Bars equal 50μm. (D) The signaling network of ARF, SLUG, SUMO1 and ECadherin for prostate cancer progression.