



# Adenoviral E2F1 And Antisense MDM2 Sensitize Prostate Cancer Cells To Androgen Deprivation and Radiation

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## Purpose/Objective:

E2F1 is a transcription factor that when overexpressed sensitizes many tumor cell types to radiation (RT). Recently, we reported that E2F1 overexpression using an adenoviral vector (Ad-E2F1) significantly radiosensitized p53-wild-type and p53-null prostate cancer cells. We also have described that MDM2 suppression via antisense MDM2 (AS) sensitizes androgen sensitive prostate cancer cells to both RT and androgen deprivation (AD).

The hypothesis here is that by targeting E2F1 and MDM2 there will be a supra-additive cell killing in response to AD, RT and AD±RT.

## Methods :

LNCAp cells were grown for 3 days, infected with Ad-E2F1 or adenoviral-luciferase (Ad-Luc; control vector) at 10 MOI for 1 hr and incubated with 200 nM AS-MDM2 or mismatch (MM; control oligonucleotide) for 48 hrs. Radiation (5 Gy) treatment was added 24 hours after AS or MM. Androgen deprivation was achieved by culturing the cells in medium containing charcoal-stripped serum (AD-medium) for 3 days prior Ad-E2F1 treatment. Apoptosis was measured by caspase 3+7 (fluorometric) and TUNEL (FACS) assays 24 hr after RT. Overall cell death was assessed by plating for clonogenic survival immediately after RT.

## Results:

Table 1. shows that when Ad-E2F1 combined with AS there is an increase in apoptosis by caspase 3+7 measurements compared to mismatch controls. Early apoptotic cell death was significantly enhanced when Ad-E2F1 and AS-MDM2 were used with AD. Additional cell killing was observed when all of the treatment groups (Ad-E2F1+AS+AD+RT) were combined. Moreover, the addition of RT enhanced the apoptotic response to E2F1+AS in all prostate cancer cell lines (LNCAp, LNCAp-RES and PC3) studied (Table 1, 2 and 3) both by caspase 3+7 and TUNEL (not shown) assays.

Figure 1. shows western blot analysis after treating the cells with Ad-E2F1, AS and/or MM. E2F1 was overexpressed and MDM2 was downregulated, as expected. Also the levels of p53 and phospho-p53 (ser<sup>15</sup>) increased after Ad-E2F1+AS-MDM2 combined treatment. The other markers that were upregulated included p21, BCL2 and RB

after Ad-E2F1+AS-MDM2 treatment. By clonogenic assay Ad-E2F1 in combination with AS-MDM2 and RT clearly reduced the survival in LNCAp cells compared to MM controls. Our data suggest that Ad-E2F1 combined with AS-MDM2 significantly enhances the response of prostate cancer cells to both androgen deprivation and radiation therapy.

Table 1. Apoptosis From Ad-E2F1+AS+AD+RT in LNCAp Cells

Groups	LNCAp		LNCAp-AD		LNCAp+RT		LNCAp-AD+RT	
	M ± SEM	p*	M ± SEM	p*	M ± SEM	p*	M ± SEM	p*
Ad-luc	225 ± 19*	-----	174 ± 13.6*	-----	327 ± 33*	-----	172 ± 29*	-----
Ad-luc+MM	348 ± 42	1.000	565 ± 79.4	0.005	525 ± 65	1.000	563 ± 64	0.017
Ad-luc+AS	439 ± 69	1.000	674 ± 30.5	0.370	638 ± 86	1.000	756 ± 134.5	0.203
Ad-E2F1	466 ± 50**	1.000	609 ± 11.4**	0.590	746 ± 128**	1.000	916 ± 115.4**	0.289
Ad-E2F1+MM	373 ± 47	1.000	795 ± 87.9	0.134	702 ± 75	1.000	845 ± 104.5	0.632
Ad-E2F1+AS	689 ± 46 <sup>§</sup>	0.002	1066 ± 133.0 <sup>§</sup>	0.036	1171 ± 90 <sup>§</sup>	0.006	1453 ± 138.8 <sup>§</sup>	0.001
AS	454 ± 60	0.044	730 ± 121.9	0.012	605 ± 66	0.0001	641 ± 81.3	0.0001

LNCAp or LNCAp-AD cells were treated with Ad-E2F1 alone or in combination with AS+RT. The effect on cell apoptosis measured by the Caspase 3+7 and TUNEL assays. Ad-E2F1 combined with AS-MDM2 resulted in significant apoptosis. However, Ad-E2F1 when combined with AS+RT with androgen deprivation resulted in significantly higher apoptosis. Similar results were observed with TUNEL assay. p\* Compared to group above, One way ANOVA, least significant difference (LSD) Test. The data shown represents the average values (± SEM) for more than 3 independent experiments.

Other Anova comparisons: p\* < 0.05

\*Ad-E2F1 vs. Ad-Luc; \*\*Ad-E2F1-AS vs. Ad-Luc+AS; <sup>§</sup>Ad-E2F1 vs. Ad-E2F1+AS

Table 2. Apoptosis From Ad-E2F1+AS+RT in RES Cells

Groups	RES		RES + RT	
	M ± SEM	p*	M ± SEM	p*
Ad-luc	105 ± 3.0*	-----	163 ± 15.7*	-----
Ad-luc+MM	132 ± 17.6	1.000	230 ± 48.1	1.000
Ad-luc+AS	182 ± 23.9	1.000	200 ± 40.7	1.000
Ad-E2F1	165 ± 12.9**	1.000	307 ± 27.6**	1.000
Ad-E2F1+MM	178 ± 9.7	1.000	299 ± 34.9	1.000
Ad-E2F1+AS	379 ± 41.3 <sup>§</sup>	0.0001	759 ± 63.9 <sup>§</sup>	0.0001
AS	195 ± 26.0	0.0001	254 ± 70.3	0.0001

RES cells were treated with Ad-E2F1 alone or in combination with AS+RT. The effect on cell apoptosis measured by the Caspase 3+7 and TUNEL assays. Ad-E2F1 combined with AS-MDM2 resulted in significant apoptosis. Ad-E2F1+AS+RT resulted in significantly higher apoptosis compared to any other groups studied. Similar results were observed with TUNEL assay.

p\* Compared to group above, One way ANOVA, least significant difference (LSD) Test. The data shown represents the average values (± SEM) for more than 3 independent experiments.

Other Anova comparisons: p\* < 0.05

\*Ad-E2F1 vs. Ad-Luc; \*\*Ad-E2F1-AS vs. Ad-Luc+AS; <sup>§</sup>Ad-E2F1 vs. Ad-E2F1+AS

Table 3. Apoptosis From Ad-E2F1+AS+RT in PC3 Cells

Groups	PC3		PC3 + RT	
	M ± SEM	p*	M ± SEM	p*
Ad-luc	125 ± 25.0*	-----	281 ± 38.4*	-----
Ad-luc+MM	144 ± 5.9	1.000	310 ± 41.8	1.000
Ad-luc+AS	142 ± 30.7	1.000	297 ± 43.7	1.000
Ad-E2F1	242 ± 49.1**	0.611	343 ± 42.3**	1.000
Ad-E2F1+MM	187 ± 23.1	1.000	249 ± 24.1	1.000
Ad-E2F1+AS	403 ± 35.2 <sup>§</sup>	0.002	633 ± 36.7 <sup>§</sup>	0.0001
AS	169 ± 8.9	0.001	278 ± 16.8	0.0001

PC3 cells were treated with Ad-E2F1 alone or in combination with AS+RT. The effect on cell apoptosis measured by the Caspase 3+7 and TUNEL assays. Ad-E2F1 combined with AS-MDM2 resulted in significant apoptosis compared to mismatch controls. Ad-E2F1+AS+RT resulted in significantly higher apoptosis compared to any other groups studied. Similar results were observed with TUNEL assay.

p\* Compared to group above, One way ANOVA, least significant difference (LSD) Test. The data shown represents the average values (± SEM) for more than 3 independent experiments.

Other Anova comparisons: p\* < 0.05

\*Ad-E2F1 vs. Ad-Luc; \*\*Ad-E2F1-AS vs. Ad-Luc+AS; <sup>§</sup>Ad-E2F1 vs. Ad-E2F1+AS

Figure 1. Effect of Ad-E2F1+AS+RT on Protein levels in Prostate Cancer cells

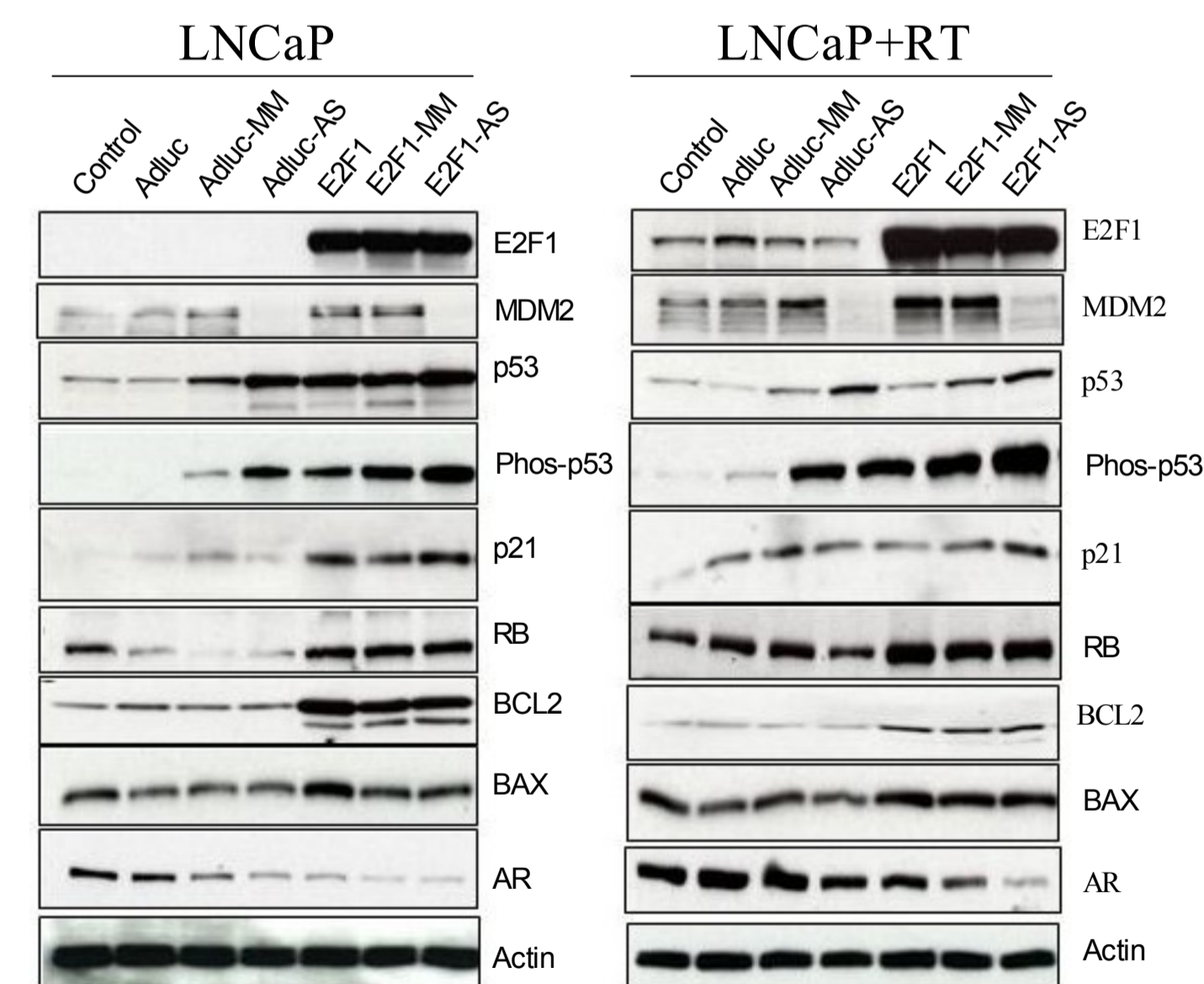
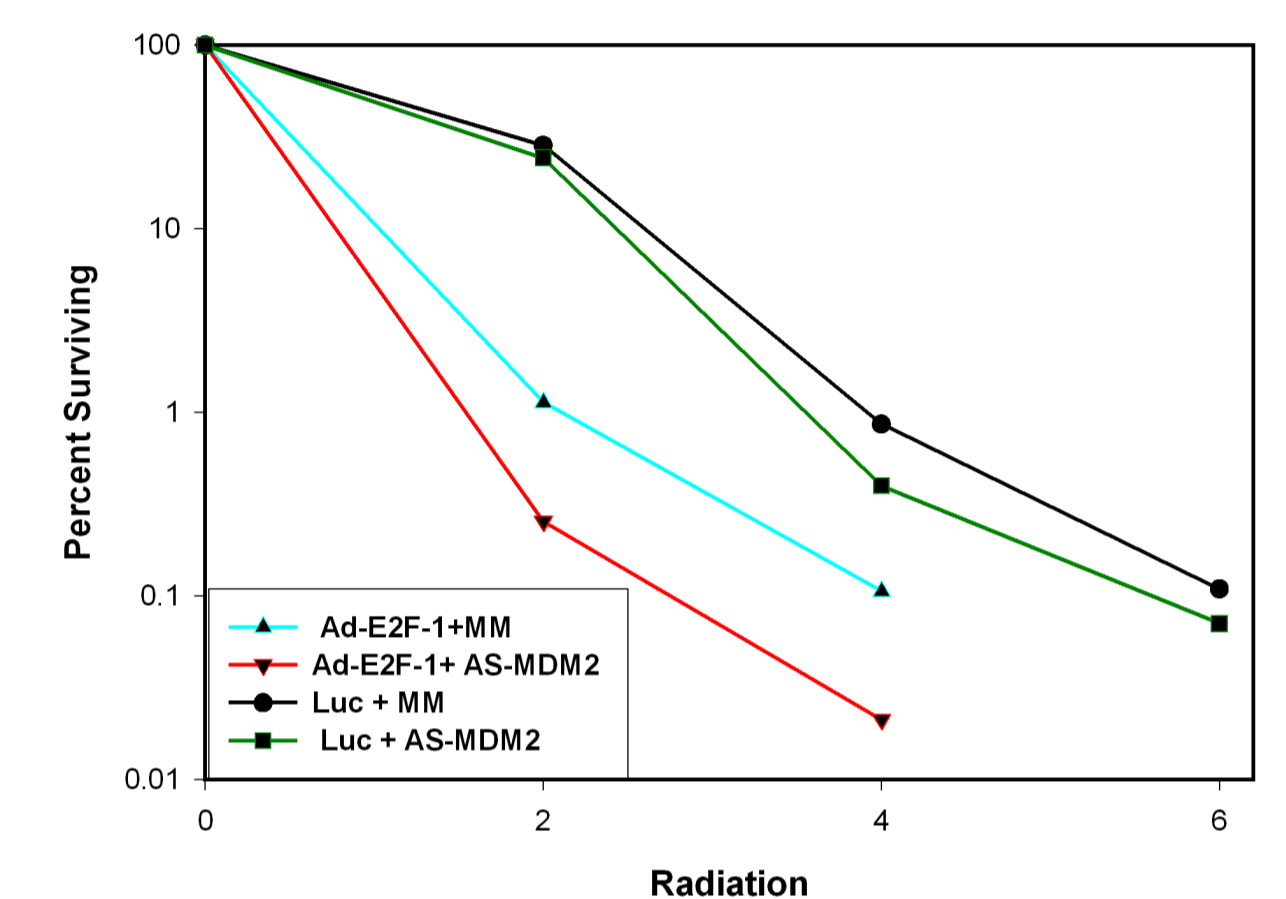


Figure 1. shows effect of Ad-E2F1+AS+RT on protein levels in prostate cancer cells LNCAp Western blots. LNCAp cells were transfected with 10 MOI of Ad-E2F1 or Ad-Luc for 1 hr, followed by 200nM of AS-MDM2 for 24 hrs. After 24 hrs of gene transduction, cells were irradiated (LNCAp+RT) 5 Gy and lysed 24 hr later. The results showed E2F1 overexpression and MDM2 suppression in LNCAp cells. Total p53 as well as phosphorylated (ser<sup>15</sup>) p53 was upregulated in Ad-E2F1+AS-MDM2+RT group compared to other treatment groups studied.

Figure 2. Effect Of Ad-E2F1 + AS-MDM2 On Clonogenic Survival Of LNCAp Cells



The clonogenic survival of LNCAp cells was significantly reduced when incubated with Ad-E2F1 (10 MOI) + ASMDM2 at 200 nM for 24 hr prior to exposure to radiation (2, 4 or 6 G). An average of 3 experiments for each cell type is shown.

## Summary/Conclusions:

- In this study we demonstrated using caspase 3+7, TUNEL and clonogenic assay that Ad-E2F1 is a radiosensitizer by itself and an increased radiosensitivity is observed when combined with AS-MDM2.
- Also our results suggest that Ad-E2F1 combined with AS-MDM2 significantly enhances the response of prostate cancer cells to both androgen deprivation and radiation therapy. By attacking the apoptotic pathway at multiple levels, the cell death response to RT±AD is compounded.
- The combination Ad-E2F1 and AS-MDM2 have potential in the radiotherapeutic management of prostate cancer.