Purpose/Objective(s): Ectopic overexpression of E2F1 via an adeno-viral vector has been shown to induce apoptosis in cancer cells. Our earlier studies show that E2F1 overexpression sensitizes prostate cancer cells to radiation in vitro and in vivo. However, E2F1 overexpression induces proliferation and may potentiate transformation. To address this concern, a truncated version, E2F1[108], without the DP1, DNA, RB binding domains was constructed. E2F1[108] retains the binding domain for S-phase kinase associated protein 2 (SKP2), an F-box protein that degrades cell-cycle regulators and proapoptotic factors (FOXO) by ubiquitin dependent mechanism. We hypothesize that E2F1[108] induces apoptosis by competitively binding to SKP2 and inhibiting its ubiquitin ligase function, leading to FOXO transactivation and upregulation of proapoptotic downstream targets, such as FasL and TRAIL.

Materials/Methods: LNCaP (p53 wild-type, AR +ve) and PC3 (p53 null, AR null) cells were infected with adenovirus-E2F1 (Ad-E2F1), Ad-E2F1[108] or Ad-Luciferase (Ad-Luc; control vector). Expression of E2F1[108] and SKP2 interacting proteins was measured by Western analysis. Immunoprecipitation was used to establish E2F1[108] binding to SKP2. Apoptosis was measured via caspase-3/7 and TUNEL assays, and immunohistochemical staining of γH2AX. Reporter assays for TRAIL, FasL and PCNA were done using a dual luciferase assay. Statistical significance was determined by analysis of variance.

Results: Ad-E2F1[108] treatment did not result in PCNA transactivation like wild-type E2F1; however, like Ad-E2F1, Ad-E2F1[108] caused significantly increased apoptosis over the Ad-Luc control by caspase-3/7 and TUNEL assays. Apoptosis was further enhanced significantly when combined with 5 Gy single dose radiation. By clonogenic assay Ad-E2F1[108] in combination with radiation was shown to reduce cell survival. Western analysis revealed that FOXO3a, BAX, BIM, p27, FasL, TRAIL and γH2AX are upregulated in E2F1 and E2F1[108] overexpressing cells. Immunoprecipitation confirmed SKP2 binding with E2F1 and E2F1[108]. Luciferase reporter assays showed that E2F1 and E2F1[108] caused an increase in FOXO3a transactivation of FasL and TRAIL; this was mimicked by siRNA knockdown of SKP2. Moreover, FOXO3a transactivation function was abrogated by overexpression of SKP2 via cDNA transient transfection.

Conclusions: This is the first report describing the radiosensitization of prostate tumor cells with an E2F1 truncated mutant that is devoid of proliferative activity. Our results indicate that E2F1[108] sequestered SKP2, promoting the stability of FOXO3a protein and consequently the transactivation of TRAIL and FasL, promoting apoptosis.

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