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Ectopic expression of the CDK inhibitor p21^{Cip1} enhances deregulated E2F activity and increases cancer cell-specific cytotoxic gene expression mediated by the ARF tumor suppressor promoter

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ABSTRACT

In cancer treatment, specifically targeting cancer cells is important for optimal therapeutic efficacy. One strategy is to utilize a cancer specific promoter to express a cytotoxic gene or a viral gene required for replication. In this approach, the therapeutic window is dependent on the relative promoter activity in cancer cells versus normal cells. Therefore, a promoter with optimal cancer cell-specificity should be used. The tumor suppressor ARF promoter, which specifically responds to deregulated E2F activity, is a potent candidate. Defects in the RB pathway resulting in deregulated E2F activity are observed in almost all cancers. Furthermore, the ARF promoter exhibits greater cancer cell specificity than the E2F1 promoter and consequently, adenovirus expressing *HSV-TK* under the control of the ARF promoter (Ad-ARF-TK) has more selective cytotoxicity in cancer cells than the analogous E2F1 construct. Ideally, cancer specific gene expression driven by the ARF promoter could be enhanced for optimal therapeutic efficacy, with minimal side effects.

We show here that ectopic expression of the CDK inhibitor p21^{Cip1} enhanced deregulated E2F activity and pro-apoptotic E2F target gene expression in cancer cells. Moreover, ectopic expression of p21^{Cip1} augmented cancer specific cytotoxicity of Ad-ARF-TK, and apoptosis induced by p21^{Cip1} was dependent on deregulated E2F activity. These results suggest that p21^{Cip1} specifically enhances deregulated E2F activity and that a combination of the CDK inhibitor with Ad-ARF-TK could be effectively employed for cancer therapy.

1. Introduction

In cancer treatment, specifically targeting cancer cells is important for optimal therapeutic efficacy. As one of the promising candidates, cancer specific expression of cytotoxic genes (suicide gene therapy) [1] or a viral gene required for viral replication (oncolytic virotherapy) [2] utilizing a cancer specific promoter is a focus of interest. By regulating a suicide gene such as *HSV-TK* or a pro-apoptotic gene such as *Bax* with cancer specific promoters, the gene is expressed specifically in cancer cells resulting in selective cell death [3]. Alternatively, by regulating a viral gene required for viral replication under the control of such promoters, cell lysis is induced by viral replication in a cancer cell-specific manner [4]. In these approaches, the therapeutic window, comprised of the balance of response versus side effects, is dependent on the relative promoter activity in cancer and normal cells. The higher the cancer cell-specificity of the promoter, the higher the relative therapeutic effects of the treatment to side effects. Furthermore, to obtain high therapeutic indices for a broad spectrum of cancers, the promoter should exhibit high activity in a wide variety of cancer cells.

In contrast to tissue specific promoters such as alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA) and prostate-specific antigen (PSA) promoters, the E2F1 promoter, which is activated by defect in the retinoblastoma (RB) pathway, displays

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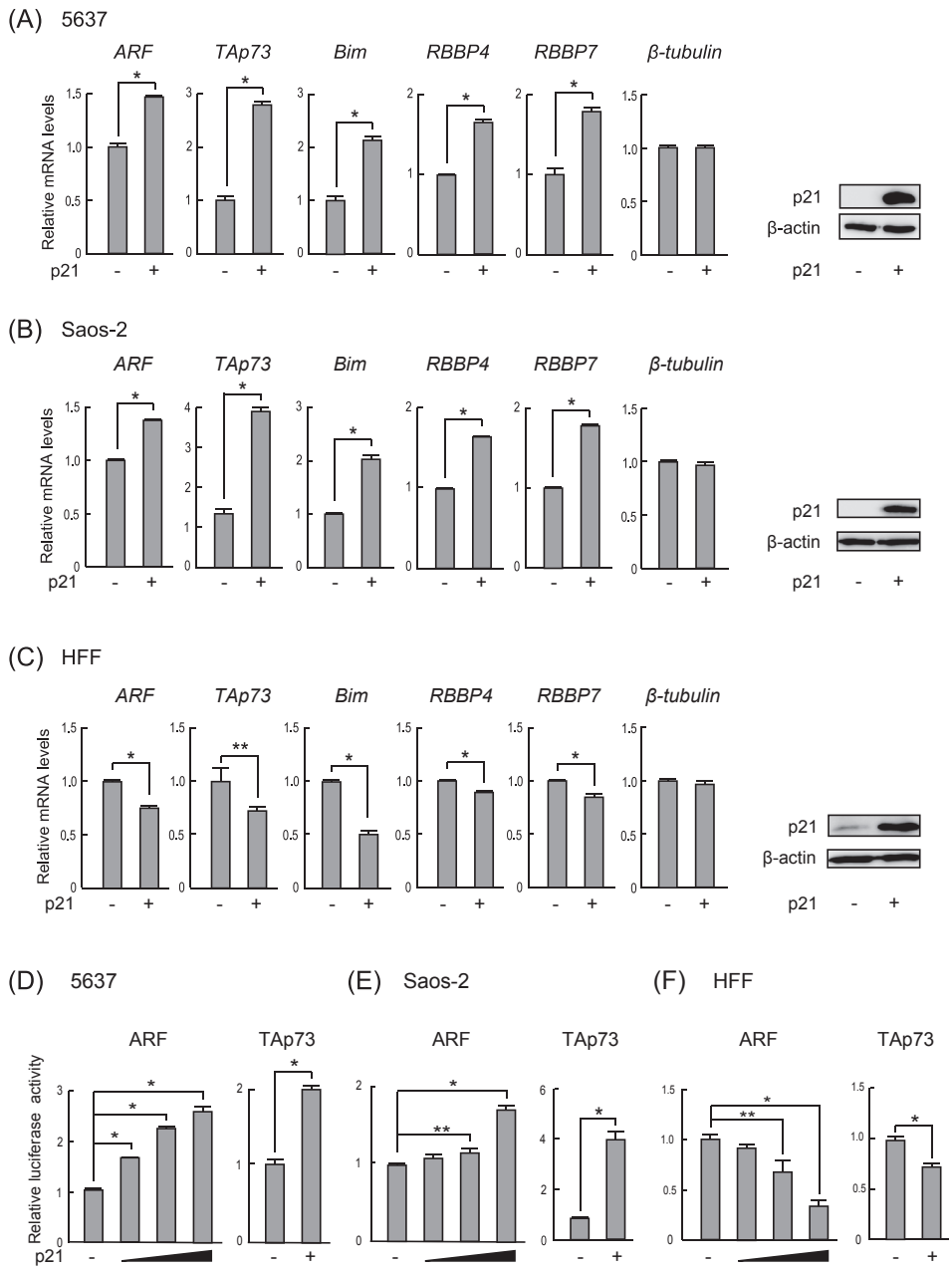

Fig. 1. Ectopic expression of $p21^{Cip1}$ activates deregulated E2F target genes specifically in cancer cell lines. (A–C) 5637, Saos-2 and HFFs cells were infected with Ad-21 at MOI 100. The cells were cultured for 24 h after infection and were harvested. The expression levels of the *ARF*, *TAp73*, *Bim*, *RBBP4*, *RBBP7*, *β-tubulin* and *α-tubulin* genes were examined by qRT-PCR, normalized to that of *α-tubulin*, and presented as relative mRNA levels. Expression of $p21^{Cip1}$ and β-actin as an internal control was examined by Western blot analyses. (D–F) 5637, Saos-2 and HFFs were transfected with pARF-Luc(-736) or p73(-892)–Luc reporter plasmid along with expression vector for $p21^{Cip1}$ and harvested 24 h after transfection. $^{*}p < 0.01$, $^{**}p < 0.05$.

strong activity in a wide variety of cancers [5]. The RB and p53 tumor suppressor pathways are impaired in most cancers [6]. The RB pathway is composed of the tumor suppressor pRB and upstream regulators such as the Cyclins/Cyclin dependent kinases (CDKs) and the CDK inhibitors $p16^{INK4a}$ and $p21^{Cip1}$. pRB binds to and suppresses transcriptional activity of E2F, an essential inducer of the G1-S phase transition. $p16^{INK4a}$ binds to and inhibits Cyclin D/CDK4 and 6, which phosphorylate and inactivate pRB. Defects in the RB pathway such as deletion or mutation of pRB or $p16^{INK4a}$, overexpression of cyclin D1 or mutation of CDK4, rendering it insensitive to CDK inhibitors, are detected in almost all cancers. Consequently, pRB is functionally inactivated and E2F activity is enhanced through deregulation from pRB. Expression of the *E2F1* gene is regulated by E2F itself [7], resulting in high E2F1 promoter activity in a wide variety of cancers. Whilst these observations support use of the E2F1 promoter to mediate cancer specific gene expression, E2F is physiologically activated by growth stimulation [7] and the E2F1 promoter is also highly active in normal growing cells [8]. Hence, the cancer specific approach with E2F1 promoter affects normal growing cells. Therefore, a more selectively active and highly cancer cell-specific promoter is needed for therapeutic interventions.

In contrast to the E2F1 promoter, which is activated by E2F induced by both growth stimulation and loss of pRB function, the tumor suppressor ARF promoter is specifically activated by E2F induced by dysfunction of pRB [9]. E2F exhibits differential

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