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ADENOVIRAL E1A: EVERLASTING TOOL, VERSATILE APPLICATIONS, CONTINUOUS CONTRIBUTIONS AND NEW HYPOTHESES

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1. ABSTRACT

Adenoviral E1A is an indispensable protein for virus-host interaction. To provide a suitable environment for viral replication, E1A physically interacts with multiple cellular proteins to reprogram gene expression and other processes of the host cells. Proteins targeted by E1A include the pRb family of pocket proteins, p300/CBP, cyclin/Cdk, the carboxyl terminal binding protein (CtBP), transcriptional regulator YY1, and the recently identified RACK1 and SWI/SNF complex. Reprogramming activity of E1A and the host cell response to this reprogramming lead to transformation, growth arrest or apoptosis. Based on the ability of E1A to override the fundamental controls of host cells, E1A has been being utilized to make continuous contributions not only to a better understanding of the molecular mechanisms underlying the regulation of transcription, cell division, apoptosis and tumorigenesis but also to new therapeutics such as gene therapy.

2. INTRODUCTION

Located at the left end of the adenovirus genome, early region 1A is the first unit transcribed after viral infection (1, 2). Two major products are translated from the transcripts and are named 289R E1A and 243R E1A, according to the numbers of amino acid residue (3). Three regions are highly conserved among various serotypes of adenoviruses and are named conserved region 1, 2, 3 (CR1, CR2 and CR3). CR1 and CR2 are shared by 243R E1A and 289R E1A while CR3 is unique for 289R E1A (Figure 1). Both 243R and 289R forms of E1A are capable of promoting virus replication, transforming rodent cells, regulating gene expression, and inducing apoptosis. Because of its ability to reprogram multiple cellular processes, 243R E1A has become an enduring tool with versatile applications and has been making new contributions continuously to our understanding of the molecular basis of cellular events.

3. ADENOVIRAL 243R E1A, A TOOL TO IDENTIFY REGULATORY PROTEINS

Since the observation of its ability to interact with cellular proteins (4, 5), E1A has been utilized as a powerful tool to identify important cellular regulatory proteins through their interaction with E1A (Figure 1). Initially observed major protein factors interacting with E1A have apparent molecular masses of 33, 60, 105, 107, 130, 300, and 400-kDa. The 33-kDa protein with histone kinase activity has been shown to be the cyclin-dependent kinase 2 (Cdk2), which is closely related to Cdc2 (6, 7). Both Cdc2 and Cdk2 are important modulators of the cell division cycle (8). The 60-kDa protein was identified as cyclin A, the regulatory subunit for Cdc2 and Cdk2 (8). The 105-kDa protein turned out to be pRb, the prototype tumor suppressor that is lost in retinoblastoma (9,10). The 107-kDa is structurally related to pRb and has similar functions

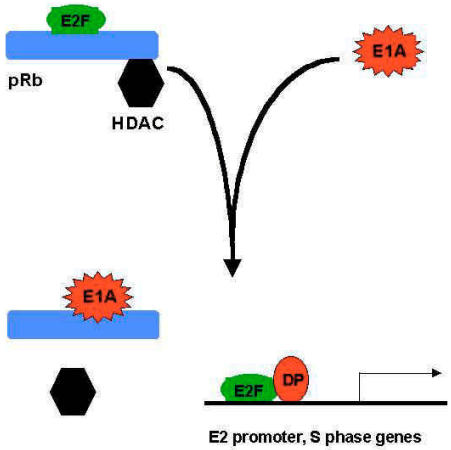

Figure 1. Schematic structures of 289R and 243R-E1A. Three regions of E1A are highly conserved between different serotypes. E1A regions required for various functions are indicated. The ellular proteins interacting with 243R-E1A are listed in the lower part. The conserved region 3 is unique for 289R E1A but is not involved in transformation, apoptosis and transcriptional repression.

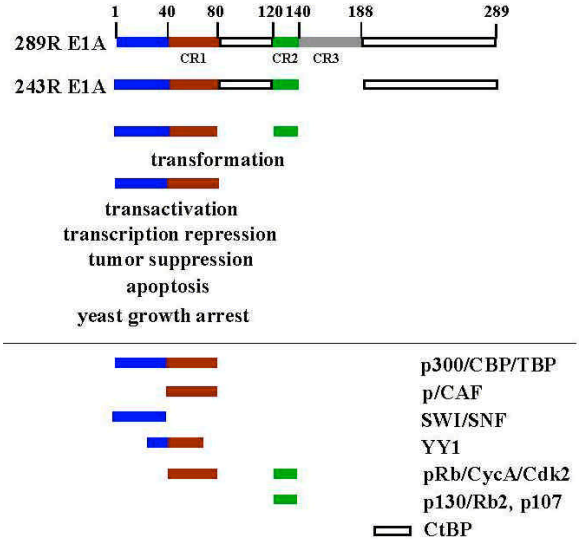

Figure 2. Mechanism underlying E1A-mediated stimulation of S-phase genes. pRb family proteins bind E2F and recruit histone deacetylases. E1A targets the pocket proteins and releases E2F, thus E2F forms heterodimers with DP1 or DP2 to transactivate S-phase specific genes and to promote DNA synthesis.

as pRb (11, 12). The 130-kDa protein is another pRb-related growth suppressor (pRb2) with extensive homology with p105 and p107, especially at the so-called pocket region (13, 14). Thus p105, p107 and p130/Rb2 form a growth suppressor family and share similar functions such as repressing E2F family transcriptional factors and interacting with histone deacetylase. Molecular cloning of the 300-kDa protein reveals it to be related to the CREB (cAMP responsive element binding protein) binding protein, CBP (15-18). Both CBP and p300 are coactivators for multiple transcriptional factors with intrinsic histone acetyl transferase activity and directly targeted by E1A (16-20). Using E1A as bait in yeast two-hybrid system to fish for new interacting proteins resulted in the discovery of CtBP, a general transcriptional co-repressor (21).

4. TARGETING SWI/SNF: MODULATING CHROMATIN STRUCTURE

The story has not stopped here. Additional proteins were found to interact with the amino terminus of E1A in a p300-independent manner (22). In addition, E1A functionally represses SWI/SNF activity, an activity required for chromosome remodeling in yeast (23). Because SWI/SNF is highly conserved between yeast and mammals (24), it was proposed that the amino terminus of E1A was able to target components of SWI/SNF complex in human cell (22). This hypothesis was first partially confirmed by the identification of E1A-associated p270 as a component of the SWI/SNF complex (25). Recently, a protein complex interacting with the amino terminus of E1A has been identified as part of the human SWI/SNF complex (26), further substantiating the notion that E1A uses its amino terminus to directly target SWI/SNF complex, thus regulating gene expression by altering chromatin structure.

5. E1A-MEDIATED TRANSACTIVATION

Another line of exploration followed the clue of E1A-mediated regulation of gene expression. An early observation that E1A promoted the viral gene expression initiated this line of investigation. These studies initially identified a cellular factor that bound to the E2 promoter and was responsible for activating this promoter, thus termed E2F (27). Later, this factor was cloned as a pRb-binding protein and turned out to be an important factor in the control of DNA replication and cell proliferation (28-30). Now E2F has exploded to a family of transcription factors including at least five members and it is clear that E1A promotes viral and host gene expression partially through stimulating E2F activity (31). The general mechanism can be outlined as E1A directly targets the pocket proteins, and releases E2F from the pRb family-mediated repression (31, 32 and Figure 2). There are some exceptions. One exception is the modulation of YY1 activity. E1A functionally and physically interacts with YY1 and releases YY1-mediated transcriptional repression (33, 34). Another exception is the regulation of proliferating cell nuclear antigen (PCNA), a factor involved in DNA synthesis and DNA repair (8). Investigations targeted at the E1A responsive element of the human *PCNA* promoter revealed the involvement of RFX1/EF-C and ATF-1 (35). In addition, the human cdc2 gene has been identified as a transcriptional target of E1A, and in this case, CBF/NF-Y and an unidentified 110-kDa protein are involved (36).

6. E1A-MEDIATED TRANSCRIPTIONAL REPRESSION

More interestingly, E1A is not a DNA binding protein and functions as a transcriptional inhibitor of multiple host genes. The function of transcriptional

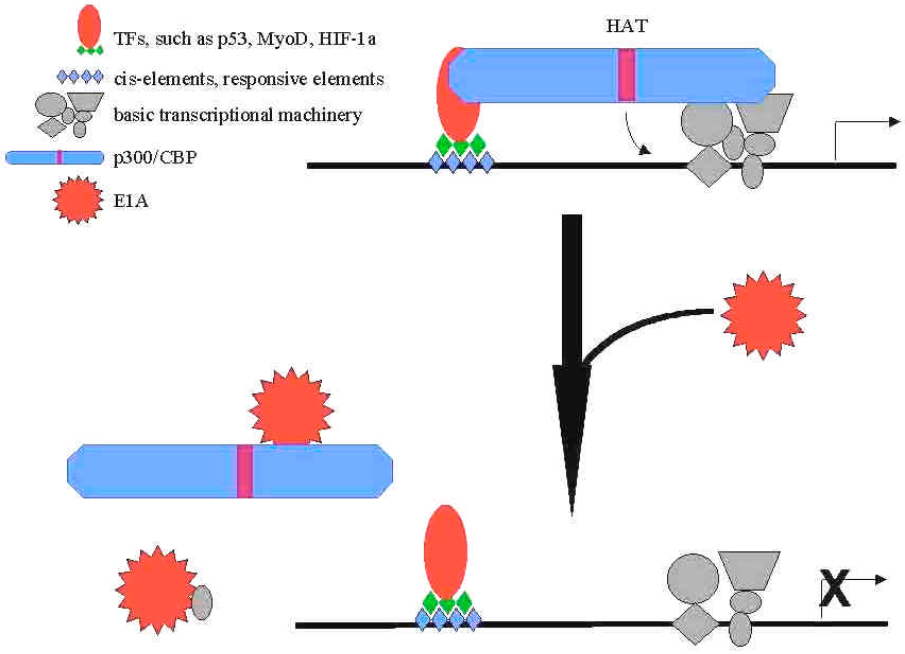

Figure 3. E1A-mediated repression of p300/CBP-dependent transactivation. Transcriptional factors such as p53, MyoD and HIF-1 require p300/CBP for maximal activity. E1A sequestrates p300/CBP and inhibits the transactivation. In addition, E1A also sequestrates TBP and p/CAF, further repressing the gene expression.

repression requires the intact amino terminus and CR1 of E1A. When fused to a DNA binding domain, however, the amino terminus and CR1 show potent transactivation activity and this activity is well conserved from yeast to mammals (37, 38). Based on this fact, it has been found that this region directly binds TBP, an important component of TFIID (38), and p/CAF, another acetyltransferase that interacts with p300/CBP (39, 40). By direct interaction with p/CAF, E1A regulates histone acetyltransferase activity of p/CAF. Therefore, sequestration of p300/CBP, TBP and p/CAF constitutes the major mechanism underlying E1A-mediated transcriptional repression (Figure 3).

Because E1A sequesters p300/CBP, and p300/CBP are cofactors for many transcription factors, E1A and special E1A mutants that cannot interact with p300/CBP have been used as simple but reliable reagents to test the requirement of p300/CBP for transcription factors of interest. The activity of MyoD, for example, is repressed by E1A but not by E1A mutant defective in binding to p300/CBP. This observation leads to the finding that p300/CBP are cofactors for MyoD and are essential for cell cycle exit and myogenesis (41, 42). Similarly, p53 activity was found to be repressed by E1A but not by E1A mutant (43). It has been confirmed that p300/CBP directly interact with p53 as cofactors and acetylate p53 at carboxyl terminus to enhance p53 transactivation activity (44-46). Another example is hypoxia inducible factor-1 (HIF-1), a transcriptional activator that mediates hypoxic response of genes coding for glycolytic enzymes, erythropoietin (EPO) and vascular endothelial growth factor (VEGF). Hypoxia-induced transcription from the EPO or VEGF promoter has been shown to be specifically enhanced by ectopic expression of p300 but inhibited by E1A (47, 48; Sang and Caro, unpublished data). The interaction between the alpha subunit of HIF-1 (HIF-1α) and p300 involves the C/H1 region of p300 and the C-terminal domain of HIF-1α, and this interaction facilitates the recruitment of basic transcriptional machinery and other coactivators such as SRC-1 to form a fully active transcriptional complex (49). These are just a couple of examples, and the list of transcription factors that require recruitment of p300/CBP for complete activation will be very long, if all related reports are collected.

7. E1A-MEDIATED APOPTOSIS AND SENSITIZATION

Expression of E1A is apoptotic in certain mammalian cells (50). While the mechanism underlying E1A-induced apoptosis is not fully understood, both p53-dependent and independent mechanisms have been suggested (51). In addition, expression of E1A confers high sensitivity to DNA-damaging agents on tumor cells in a p53-independent manner (52). Interestingly, exposure of cells to DNA damaging agents induces growth arrest accompanied by expression of a spectrum of genes that encode enzymes involved in glucose metabolism and are transcriptional targets of HIF-1 (Zhou, personal communication). Furthermore, non-genotoxic agents that induce apoptosis down-regulate enzymes involved in glucose metabolism (53). In addition, it is reported that Akt/PKB protects cells from apoptosis by promoting glycolysis (54, 55). Because HIF-1 is able to change the glucose and oxygen metabolism and is usually activated in

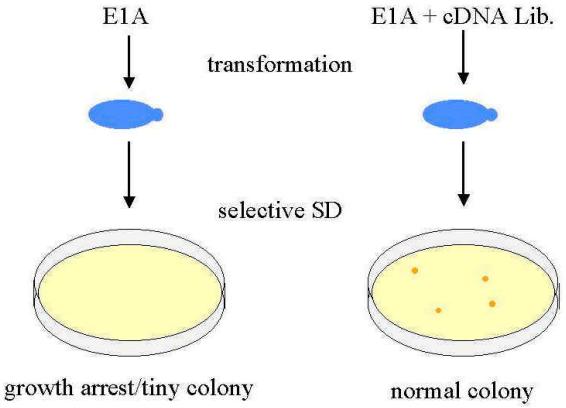

Figure 4. Strategy of functional screening by rescuing yeast growth. Expression of proteins such as E1A in mammalian cells leads to apoptotic or toxic effects to the cells. However, expression of these proteins in yeast may lead to growth arrest or slow-growing phenotype. When the apoptotic gene is co-transformed with an expression library into yeast, fast-growing colony may carry a cDNA encoding a protein that antagonizes the apoptotic protein, thus rescuing the yeast growth.

non-hypoxic tumor cells, we hypothesize that HIF-1 may confer an activity facilitating cell adaptation not only to the hypoxic stress but also to other apoptotic stresses. Thus repression of HIF-1 activity may play a role in E1A-mediated apoptosis and sensitization.

While yeast does not undergo apoptosis, expression of E1A in some strains of *S. cerevisiae* leads to growth arrest or a slow-growing phenotype, and the domain requirement for growth arrest or slow-growing phenotype in yeast is identical to that for apoptotic effects in mammalian cells (56). Based on this phenomenon, a functional screening strategy has been designed and employed to seek mammalian proteins that can functionally interact with E1A and thus rescuing the slow-growing phenotype in yeast (Figure 4). This strategy has identified RACK1, standing for *R*eceptor for *A*ctivated *C*-type protein *K*inase, as an antagonizer of E1A activity in yeast and in mammalian cells (56). Because mutation of cpc2, the yeast counterpart of RACK1, leads to growth arrest and because expression of RACK1 is able to rescue the growth arrest (57), it is reasonable to propose that E1A blocks a growth-related function of cpc2/RACK1. While the precise function of RACK1 involved in growth control still needs to be investigated, E1A exemplifies a novel strategy to study apoptotic or toxic proteins in yeast.

8. E1A-MEDIATED TUMOR SUPPRESSION AND GENE THERAPY

Since the perception that E1A may cause apoptosis and serve as a tumor suppressor (58, 59), the potential of E1A as a magic bullet to fight against cancer has been explored intensively (60, 61). Methodology such as gene therapy is being developed and tested to deliver E1A into tumor cells (62, 63) and several patents have been granted to E1A-based cancer gene therapy.

Not only is E1A a bullet for cancer but also an important player in other fields of gene therapy. In recent years, gene therapy has been gaining increasing momentum. Despite some shortcomings, viral vectors are still the majority in experimental gene therapy and clinical trials. Among the viral vectors, adenovirus-based vector is commonly used for cancer gene therapy and transient gene transfer in basic research because of its high infection rate and simplicity of packaging, preparation and preservation. Adeno-associated virus (AAV)-based vector is the most promising one in treatment of hereditary disease resulted from dysfunction of a single gene. While E1A usually is not included in the genome of recombinant adenovirus or AAV, E1A function is indispensable for the packaging of these therapeutic vectors. Thus E1A must be provided either by a trans-plasmid or by the packaging cell line, such as HEK293, which is derived from human embryonic kidney and expresses E1A constitutively. Therefore, E1A could be used to investigate the packaging processes of recombinant virus, aiming at a simple, cost-effective and highly efficient way to produce viral vectors for gene therapy.

9. PERSPECTIVE

We have summarized the major functions of the adenoviral E1A oncoprotein, the major applications of E1A in basic research and potential applications in the clinical therapy based on these functions. However, the mechanism underlying the effects of E1A on host cells and the mechanism by which the host cells respond to these effects are not completely understood. Since accumulating evidence suggests that E1A may affect the basic energy metabolism of host cells, a process involving the utilization of glucose and oxygen, we predict that further study towards this direction will help us to understand E1A-mediated apoptosis and tumor suppression. We strongly believe that E1A will continue to be a powerful tool in the dissecting of the regulation of cellular processes, as it has been for the past twenty years. In addition, the yeast rescuing strategy exemplified by E1A will prove to be a useful strategy to study other apoptotic or cytotoxic proteins in yeast (56). Finally, we conclude by stating that E1A, the enduring tool, will continue to make new contributions to both basic research and clinical therapy.

10. ACKNOWLEDGMENTS

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