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E4orf1 as a key modulator in oncogenesis and of metabolism in Adenovirus infection

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Human Adenoviruses are a diverse family of viruses that can infect a variety of tissues causing acute or persistent infection. Viruses induce numerous cellular alterations as they hijack cellular functions to promote viral progeny. Recent research has shed light on the functions of viral proteins in orchestrating viral production, revealing that many of these functions overlap with oncogenesis or metabolic disruption. Studies of the Adenovirus family (Adenoviridae) have identified oncogenic members, such as Adenovirus (Ad-)2, 5, 9, and 12, and also Ad-36, which is most extensively studied for its ability to induce metabolic alterations. Specifically, Adenoviruses encode a gene product known as early region 4 open reading frame 1 (E4orf1), which has emerged as an oncoprotein and regulator of metabolism depending on the lineage of the infected host cell. This article aims to provide insight into the functions of the viral protein E4orf1 and the overlapping similarities between the oncogenic process and cell metabolism.

KEYWORDS

Adenovirus-36, adipocytes, oncogenesis, E4orf1, metabolism

Introduction

Cancer and obesity are two leading causes of morbidity and mortality worldwide, and recent research suggest an interconnection between the two (1). The study of tumor viruses has been instrumental in advancing our understanding of molecular oncology, leading to the discovery of oncogenes and tumor suppressor genes, and the elucidation of the cellular

processes that they regulate (2). In fact, the International Agency for Research on Cancer has reported that approximately 10% of cancer cases worldwide are attributable to chronic viral infection (3).

Although direct viral-induced cancer is rare, viruses can promote oncogenic transformation through chronic infections and the resulting cellular alterations. As intracellular parasites, viruses depend on the reprogramming of cellular metabolism to facilitate their replication; this reprogramming also occurs in oncogenesis and obesity. The degree of oncogenic or metabolic alteration varies depending on the viral family and may involve direct alteration to cellular proteins or signaling pathways.

The Adenovirus (AdV) family is an example of a family of viruses that induces both oncogenic and metabolic alterations, through direct alteration and signaling pathway misregulation. Although initially thought to transform only rodent cell lines, several AdV members have been found to transform certain human primary cells (4). We focus in this article on AdVs and their ability to induce oncogenesis and metabolic alterations.

Studies mapping the oncogenic activity of AdVs have revealed that the E1 transcriptional unit is responsible for cell transformation. This unit encodes for the oncogenic proteins E1A and E1B, which must be co-expressed to achieve full transformation (5). Analysis of the E1 region has contributed enormously to the development of molecular biology, as it has shown the initial mechanisms of oncogenic transformation through the deregulation of DNA synthesis and proliferation. Through direct or indirect interactions, E1A activities lead to induction of S phase, inactivation of differentiation genes, and activation of proliferation in the host cell. The classic example of direct interaction is the deregulation of the pRb protein, whereas an example of indirect interaction is through histone acetylation of scaffolding proteins (6, 7).

Another essential mechanism for understanding cell cycle control is the inhibition of p53 by E1B to avoid apoptosis. The molecular mechanism involves suppressing p53 transcriptional activity by sequestering the protein in perinuclear bodies, inducing its degradation, or through posttranslational modifications (8, 9). The study of AdVs has also contributed greatly to the understanding of RNA splicing, a process involving a series of reactions catalyzed by a complex of small nuclear ribonucleoproteins (spliceosome) to generate a mature transcript. In the 1980s, in contrast to what was known regarding transcription in prokaryotic cells, little was known about transcription in eukaryotic cells. However, in 1977, two independent groups described the new messenger RNA (mRNA) biosynthesis mechanism using hybridization and electron microscopy assays. To date, almost 1.2 million transcripts have been described using differential RNA sequencing (dRNA-seq), making AdVs the most transcriptionally complex viruses studied in terms of splicing sites (10–12).

As previously discussed, it was initially thought that only E1 region genes were involved in oncogenic transformation. However, it was soon discovered that genes encoded in the E4 region also play a role in altering cellular mechanisms. The transformation potential is attributed to three gene products, namely E4orf1, E4orf3, and E4orf6. Although the last two have overlapping functions with E1A and E1B, and support the oncogenic process in cell models, the E4orf1 of Ad-9 operates independently of E1A and E1B functions.

In fact, E4orf1 was first identified as a new oncoprotein because its sole expression was sufficient to induce anchorage-independent growth in rat cells (13).

E4orf1 in human AdVs reprogram cellular signaling pathways in infected cells to shape an optimal microenvironment for viral replication. Although not all AdV family members possess the oncogenic capacity of E4orf1, many can deregulate crucial cellular metabolic processes that overlap with oncogenic processes. Consequently, E4orf1 has been investigated as both an oncogenic model and a potential therapeutic agent for obesity and diabetes (see Figure 1 Timeline). This mini review focuses on the roles of E4orf1 in targeting cellular proteins and its involvement in oncogenic and metabolic pathways.

The E4 region organization

The transcription of the AdV genome, on infection, depends on the sequential activation of early and late transcriptional units. There are five early units identified as E1a, E1b, E2a/b, E3, and E4, while examples of later units include IX, IV2a, and L1–L5 genes (21). Initially, the E4 unit was reported to contain eight open reading frames (orfs), encoding proteins with sizes from 11 to 34 kDa (22). However, it is now more widely accepted that E4 encodes for seven different proteins that collectively sculpt the intracellular milieu, optimal to yield viral progeny (21, 23).

Many AdVs share the same E4 unit organization, which is transcribed in the leftward direction and situated at the right end of the genome. The expression of the E4 products is regulated both transcriptionally and posttranscriptionally. The E4 promoter spans approximately 200 bp upstream from the transcription start site and contains binding sites for the viral transcription factor E1A, as well as cellular factors ATF-2 and E4F1 (24, 25).

E4 expression is also importantly regulated by alternative splicing. Initially, it was demonstrated that there are at least 10 splicing sites on E4, with some transcripts generating two or more polypeptides (26). Subsequently, the 10 splice sites were characterized either as a combination of four donor and six acceptor sites or as eight donor and two acceptor sites, encoding a total of seven unique proteins (23, 27). These seven unique products are E4orf1, E4orf2, E4orf3, E4orf4, E4orf6, E4orf6/E4orf7, and E4orf3/E4orf4, based on their orf arrangement. We focus on E4orf1 because it possesses oncogenic potential in several AdVs and has a unique ability, in Ad-36, to deregulate cellular metabolism.

Biochemical features of the E4orf1 protein

E4orf1 is a protein expressed in various AdVs, including Ad-2, Ad-3, Ad-5, Ad-9, Ad-12, and Ad-36. The E4orf1 proteins from these viruses vary from 125 to 128 amino acids in length and have sizes ranging from 14 to 17 kDa. They share 50%–65% identity and possess several potential glycosylation sites, which may contribute

to the diversity of their sizes (14, 22). E4orf1 is primarily localized in cytoplasm, where it modulates cellular signaling pathways. However, one study reported E4orf1 forming nuclear complexes and participating in gene transcription (28).

Three E4orf1 regions, termed RI, RII, and RIII, are conserved across several AdVs and have been deemed significant through mutational analysis (Figure 2) (29). The function of RI is unclear, but it appears to form a functional domain with RII. This RI–RII domain seems crucial for the transforming activity of certain E4orf1 protein members (30). RII exhibits some degree of homology with eukaryotic dUTPases, but no enzymatic activity has been demonstrated for E4orf1 so far (30). dUTPases can complex with transcription factors and inhibit their function and it is possible that E4orf1 evolved to mimic this function, as bioinformatic analysis revealed that E4orf1 shares a very similar three-dimensional (3D) structure to dUTPases (29, 30).

E4orf1 interacts with several cellular proteins through RIII, which features a C-terminal *bona fide* PDZ domain-binding motif (PBM) with the consensus sequence S/T–X/V/I (X denotes any amino acid) (31, 32). The PDZ domain of RIII is present in several AdV members, such as Ad-2, Ad-3, Ad-5, Ad-9, Ad-12, Ad-17, and Ad-36 (Figure 2). Unlike other PDZ proteins where only 10–15 C-terminal amino acid residues retain the binding activity, mutational analysis indicates that E4orf1 requires an intact 3D structure to function, as seen in Ad-9 (21, 32). No phosphorylation or other posttranslational modifications have been reported for E4orf1; however, most of the associated cellular proteins are phosphorylated. E4orf1 binds to key cellular proteins involved in signal transduction, cell proliferation, and tight junction assembly, and modulates the PI3K-AKT signaling pathway and glycolytic metabolism (17). Most cellular targets of E4orf1 are located in the cytoplasm, with MYC being the only exception. MYC is also modulated in the nucleus by a ternary complex formed by E4orf1–E4orf6–MYC, which activates their transcriptional activity (Figure 2) (28).

E4orf1 in oncogenesis

Viruses alter host cells to support the metabolic demands of their viral progeny. In persistent infections, viruses also alter the replicative and survival capacity of host cells, with resulting consequences for oncogenesis. The first cellular protein identified as a partner of E4orf1 was hDlg/SAP97 (Dlg1). Dlg1 is a protein rich in protein–protein interaction domains, containing three PDZ, one SH3, and one GuK domain. In drosophila, mutations in Dlg result in epithelial tumors exhibiting loss of apicobasal polarity, leading to the initial classification of Dlg1 as a tumor suppressor protein. Dlg1 has been associated with the development of cancer because it controls two very critical signaling pathways for cell proliferation: the Wnt pathway and the PI3K pathway. By interacting with APC (*Adenomatous polyposis coli*) tumor suppressor gene, it negatively regulates cell cycle progression toward S phase, while its interaction with PTEN regulates the PI3K pathway (33, 34).

It is important to note that the second PDZ domain, which is the target of E4orf1 interaction, also regulates the interaction with APC. Therefore, it has been proposed that E4orf1 promotes oncogenic transformation through this deregulation (32, 35). Moreover, E4orf1 forms a complex with Dlg1 using their trimerization and PMB domains, which is then utilized to recruit PI3K to the plasma membrane. This process subsequently activates AKT kinase, ultimately enhancing viral replication and inducing cell transformation (36, 37). The ability to deregulate the PI3K pathway is conserved among various AdV members, such as Ad-3, Ad-5, Ad-9, Ad-12, and Ad-36, all of which can activate the pathway in a Dlg1-dependent manner. However, Ad-36 is not classified as oncogenic despite inducing substrate-independent growth in soft agar assays. These findings show that cells have acquired the ability to avoid apoptosis and maintain constant proliferation as a consequence of providing a favorable environment for viral progeny assembly (38).

Timeline Key findings based in Oncogenicity or Metabolism by E4orf1

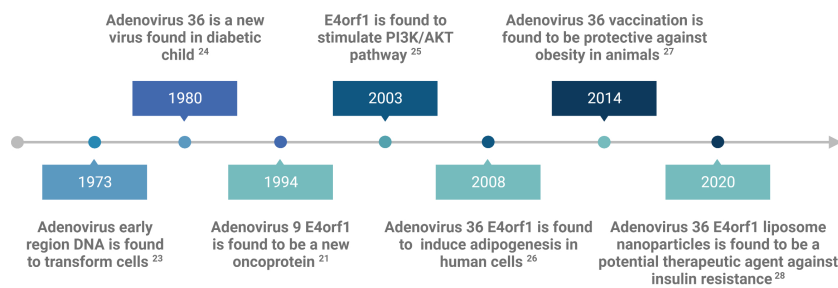


FIGURE 1
Timeline of the key events in the research of E4orf1 function (14–20).

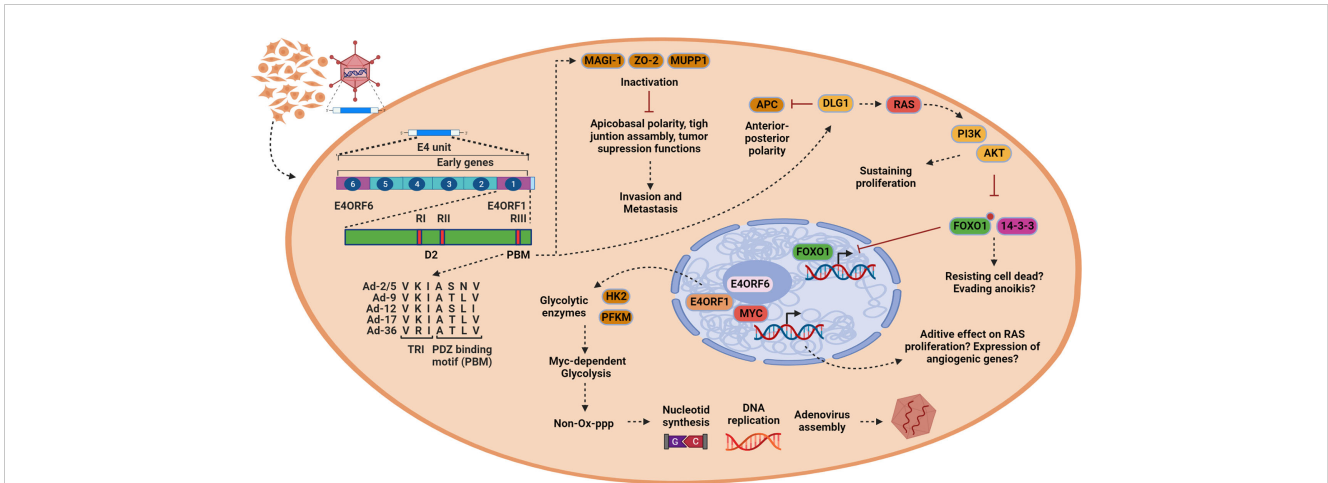


FIGURE 2

In epithelial cells, E4orf1 induces processes associated with oncogenesis and tumor progression. Once the viral genome is released (left), the transcription of the early genes (only E4 unit is indicated) begins. E4orf1 (green bar) consists of two protein domains. The first of them is designated domain 2 (D2), which is formed by the region RI and RII described initially by mutational analysis. This region shows homology with eukaryotic dUTPases that beyond their enzymatic activity can interact with transcription factors to inhibit their function. E4orf1 through this domain interacts with E4orf6 to form a complex with Myc transcription factor, but, in contrary to dUTPases, the transcriptional function of MYC is activated. The second domain is the PDZ domain-binding motif (PBM) initially called region RIII, which allows E4orf1 to interact with several cellular proteins. The trimerization element (TRI) and PBM sequence alignment is conserved among various members of the Adenoviruses (AdVs) (Ad-2/5, Ad-9, Ad-12, Ad-17, Ad-36) and is shown by an arrow from PBM in the figure (bottom left). E4orf1 dysregulates Dlg1 through the PBM and TRI element, activating a sustained proliferation through the RAS/AKT signaling pathway (upper right). Moreover, it is possible that through PI3K/AKT E4orf1 may promote cell survival by inactivating the FoxO1 transcription factor, by inactivating the pro-apoptotic protein Bad, or by inhibiting pro-caspase 9, all of which promote escape from anoikis (bottom right). However, E4orf1 dysregulates the proteins MUPP1, MAGI-1, and ZO-2. These proteins participate in modulating cell polarization and have tumor suppression activities, which can lead to cell invasiveness (top in the middle). Finally, E4orf1 forms a complex with E4orf6 through D2 domain to interact with MYC transcription factor, inducing enzymes [hexokinase 2 (HK2) and phosphofructokinase 1 (PFKM)] responsible for glycolysis (bottom in the middle). This MYC-dependent glycolysis (MDG) contributes to generating intermediary products used for nucleotide synthesis via the non-oxidative pentose phosphate pathway (PPP), resulting in efficient viral assembly. It is possible that MYC activation also has an effect on cell proliferation or the expression of angiogenic genes, but this must be verified. This figure was created with BioRender.com.

A crucial point in regulating the PI3K signaling pathway is the involvement of the phosphatase and tensin homolog (PTEN), which is often mutated in many cancers. This mutation promotes uncontrolled cell growth by prolonging PI3K signaling (39). In addition to recruiting PI3K through its interaction with Dlg1, E4orf1 might also recruit PTEN. If this were to occur, E4orf1 would ensure a state of constant proliferation without the negative feedback of PTEN. However, it has not been reported that the E4orf1/Dlg1 complex can deregulate the function of PTEN, although it is known that Dlg1 interacts with PTEN (40).

Last, further evidence that points towards Dlg1 deregulation playing a role in cancer is that Dlg1 is a target of other oncogenic viruses. For example, the Tax protein of human T-cell leukemia virus type 1 and E6 proteins from high-risk human papillomavirus (HPV) types, such as HPV-16 and HPV-18, can interact with and disrupt the function of Dlg1 (32).

A second target protein, MAGI-1, was identified following the discovery of E4orf1-mediated dysregulation of Dlg1. MAGI-1 has been implicated in regulating various processes, such as cell-cell adhesion, migration, proliferation, signaling, and survival. Like Dlg1, MAGI-1 acts as a tumor suppressor in different types of cancer, including liver, colorectal, cervical, brain, and gastric, because it also regulates crucial signaling pathways, such as PI3K/AKT and Wnt/ β -catenin. Three isoforms of MAGI-1 (a, b, and c) are generated through alternate splicing, and they are widely expressed in different tissues. Interestingly,

E4orf1 can interact with isoforms b and c via PDZ domains 1 and 3 to sequester the proteins at particular points in the cell cytoplasm, thereby inactivating their cellular functions (Figure 2) (41).

This observation aligns with the oncogenic capacity of E4orf1, as MAGI-1 also interacts with PTEN, which allows the virus to maintain a constant state of proliferation in the host cell, as mentioned above. The deregulation of PDZ proteins is a common strategy used by other pathogenic viruses, with several examples of MAGI-1 activity control being well documented. For instance, the Tax1 protein disrupts MAGI-1's function, the E6 protein sends it to degradation, and the NS1 protein of influenza seemingly regulates the antiviral response mediated by interferon through its interaction with MAGI-1 (42–44).

Another tight junction protein targeted by E4orf1 is the multi-PDZ domain protein 1 (MUPP1), which was cloned from human fetal brain cDNA library through interaction with the 5-HT2C receptor. The human gene encodes a 2054 amino acid protein with 13 PDZ domains. MUPP1 is expressed in various tissues and was initially proposed as a novel scaffold protein involved in cell signaling because of its similar structural organization with InaD from *Drosophila melanogaster*, which regulates the rhodopsin signaling pathway (45). E4orf1 sequesters MUPP1 into the cytoplasm and inactivates its function through PDZ domains 7 and 10, while the 18E6 oncoprotein inactivates its function by sending it to degradation (46).

The most likely role of this deregulation is to overcome the blockage of cell cycle progression, as some evidence suggests MUPP1 acts as a negative regulator of proliferation. E4orf1, by sequestering the protein in the cytoplasm, would circumvent this negative control. Supporting this proposal, a study using a lung cancer model showed that MUPP1 functions as a tumor suppressor by inhibiting cell proliferation through regulation of the Hippo-YAP signaling pathway (47).

However, through the same PDZ domain, deregulated by E4orf1 (PDZ10), MUPP1 seems to regulate the activity of the proto-oncogene c-Kit. *In vitro* interaction assays have shown that the constitutively active form of c-Kit does not interact with MUPP1, while the wild type does. This suggests that MUPP1 may regulate the receptor activity prior to activation with its ligand (48). This is important because c-Kit induces signaling pathways that regulate proliferation, differentiation, or metabolism, which are involved in the development of various types of tumors (49).

In addition, other evidence supporting MUPP1 as a negative regulator of proliferation has shown that the TAPP1 protein was identified as an adapter protein to recruit MUPP1 to the plasma membrane in response to signals where there are high levels of PtdIns(4,5)P₂ (50). This again coincides with the PI3K signaling pathway, as after generating PtdIns(3,4,5)P₃ for proliferative AKT signaling it is necessary to return to the basal state and generate PtdIns(4,5)P₂. This homeostasis is carried out by phosphatases such as PTEN, and it has been reported that MUPP1 interacts with PTEN through its PDZ domain. Therefore, it is conceivable that this interaction increases the concentration of phosphatase in the plasma membrane and regulates proliferative signaling.

Finally, another cell adhesion molecule deregulated by E4orf1 is ZO-2. This protein is encoded by the *TJP2* gene and can be present in both tight and adherent junctions. ZO-2 is a scaffolding protein that has three PDZ domains in its N-terminal domain, allowing it to regulate the formation of tight junctions. The protein can be found in the cytoplasm, tight junctions, or in the cell nucleus. In fact, it has been reported that in the nucleus it can act as a transcription suppressor and block proliferation by interacting with the Myc transcription factor, preventing the latter from activating one of its target genes, *CCND1* (51). E4orf1 is capable of sequestering ZO-2 in the cytoplasm, and the objective of this seems to be overcoming the cell cycle entry block induced by ZO-2 (52).

However, it has been mentioned that E4orf1 activates the AKT pathway, which has an inhibitory effect on GSK3B kinase by phosphorylating it at the Ser9 residue. GSK3B inactivation releases the β -catenin protein, which can enter the nucleus and activate proliferation through Myc expression. It is important to mention that ZO-2 prevents GSK3B phosphorylation by AKT, which in turn blocks the proliferation (53). Again, E4orf1, by sequestering ZO-2, ensures a constant proliferative state by allowing β -catenin to act in the nucleus. Therefore, ZO-2 is considered a tumor suppressor, which coincides with reports indicating that its expression is silenced in various types of cancer (54).

In normal epithelium, cells that lose contact with the extracellular matrix (EM) undergo apoptosis. This type of death is called anoikis, and its purpose is to prevent cells from proliferating

in inappropriate places (55). Physiologically, cells are protected from anoikis by signal transduction through integrins and growth factor receptors, which generally lead to activation of the PI3K/AKT pathway. Activation of this pathway promotes cell survival by inactivating the pro-apoptotic protein Bad, by inhibiting pro-caspase 9 or by inducing the nuclear exclusion of FoxO transcription factor (56–59). As mentioned previously, PI3K/AKT is the main pathway altered by E4orf1, and so it is plausible that anoikis is also deregulated in virus-infected cells. This proposal aligns with the fact that one of the most notable properties of cells expressing E4orf1 is anchorage-independent growth, a test that precisely indicates that the cells are evading anoikis in addition to acquiring the ability to constantly proliferate. Furthermore, in 3D cultures, it was shown that apoptosis is important for the formation of the lumen in glandular epithelia. Cells in contact with the EM undergo apical-basal polarization, while cells in the lumen (non-polarized and not in contact with the matrix) die by apoptosis. Finally, the proliferation of the polarized cells is suppressed to give rise to the glandular acinus (60). Therefore, tumor cells dysregulate polarization, apoptosis, and proliferation at the beginning of carcinogenesis. Returning to the proteins altered by E4orf1, several (Dlg1, MUPP1, MAGI-1, and ZO-2) participate in modulating both polarization and proliferation, explaining the oncogenic potential of the viral protein. However, we speculate that evasion of anoikis also contributes to the oncogenic potential of the viral protein, as the main pathway altered by E4orf1 (PI3K/AKT) protects against this type of cell death and there is a report of the involvement of Dlg1 in conferring resistance to anoikis (61). Overall, E4orf1 induces loss of control of cell proliferation and apoptosis, two important events at the beginning of the oncogenic process, and also induces loss of polarity that is involved in the invasiveness of tumor cells. These three tumor-acquired capabilities are considered important hallmarks of cancer (39).

E4orf1 in metabolism dysregulation

Among the members of the AdV family, Ad-36 possesses the highest correlation with obesity in both animal models and humans. Since its identification in 1980, the first report of the adipogenic potential of Ad-36 was demonstrated by infecting chickens and mice. In this study, the animals developed an increase in visceral adipose tissue and viral DNA was isolated from adipose tissue, suggesting that the principal target cells are adipocytes. Other studies indicate, however, that the virus can spread to other organs in infected animals (62, 63). In another study using monkey models, a significant association was reported between antibodies against Ad-36 and increased body weight, compared with uninfected animals, indicating the adipogenic potential of this virus (64). Moreover, in a chicken model, it was shown that Ad-36 can be transmitted horizontally and induce obesity, suggesting that transmission in humans can take place by a similar mechanism (65). Finally, various meta-analyses have demonstrated an association with the risk of developing obesity [mean odds ratio (OR) = 2.2] in infected adults, and the analysis in different countries reveals a 30% prevalence of infection among obese individuals.

Therefore, these studies in animal models and humans highlight the significant role of Ad-36 infection in altering adiposity and contributing to the development of obesity (63).

Using the 3T3-L1 pre-adipocyte cell model, it was demonstrated that Ad-36 infection has an adipogenic effect. On day 2 post infection, the virus begins its replication but is abortive; however, the mRNA expression peak of early genes (including *E4orf1*) coincides with the differentiation of adipocytes. The pharmacological inhibition of *E4orf1* expression decreases lipid accumulation and the expression of the *C/EBPβ* gene, which is important for lipogenesis, suggesting the participation of the viral protein in the induction of adipogenesis (66). Subsequently, using *in vitro* and *ex vivo* models, it was reported that the adipogenic effect of Ad-36 is partly due to the modulation of leptin expression and glucose metabolism. In both 3T3-L1 cells and in primary rat adipocytes or in their fatty tissue, it was observed that infection by Ad-36 reduces leptin expression, with the concomitant increase in glucose uptake. In turn, a progressive increase in lipid accumulation was observed due to the expression of genes involved in their synthesis, such as *FAS* and *ACCI*, revealing part of the mechanism behind the positive effect on adipogenesis (67).

To evaluate if the adipogenic effect was present in humans, experiments were conducted using primary human adipose-derived stem/stromal cells (hASC). In this model, it was observed that Ad-36 increases the expression of *PPARγ* and *C/EBPβ*, which are master regulators of adipocyte differentiation. Concurrently, an increase in fatty acid-binding protein (AP2), which is a marker of adipocyte differentiation, was observed. This was also present in LPL, which is a key enzyme for the uptake of triglyceride-delivered fatty acids to adipose tissue. Using hASC extracted from subjects infected with Ad-36, a greater accumulation of lipids was observed,

demonstrating that the virus effectively induces the commitment and differentiation toward adipocytes (68).

However, using a microarray assay and gene set enrichment analysis with a human mesenchymal stem cell model, it was confirmed that Ad-36 induces the expression of at least 35 genes involved in adipocyte differentiation, such as *LPL*, *C/EBP*, *ATF3*, and *FABP4*. Interestingly, the importance of the *PPARγ* signaling pathway was highlighted again, as genes such as *ADIPOQ*, *APOC3*, and *PLTP* were upregulated and are involving in enhancing glucose utilization and fatty acid combustion (69). Finally, in the search for the specific viral gene responsible for adipogenesis, it was found that Ad-36 does so through *E4orf1*. In both 3T3-L1 cells and hASCs infected with Ad-36, an induction of adipogenesis accompanied by cell proliferation was observed through signals mediated by the phosphorylation of AKTser473 and P38thr180. Through the expression of the recombinant protein in the 3T3-L1 cells, the adipogenic effect was confirmed, because if the expression of the viral protein is inhibited by using siRNA technology, then the adipogenic effect is reversed, showing that *E4orf1* is the viral adipogenic regulator (18). In addition, the recombinant protein induced the redistribution of FoxO1 from the nucleus to the cytoplasm in an AKT-dependent manner, which in adipocytes is important to allow their differentiation (70, 71). Overall, these studies have shown that *E4orf1* can induce hypertrophy and hyperplasia in adipocytes by increasing their capacity to store glucose and induce proliferative signaling pathways (Figure 3).

Molecularly, the metabolic deregulation of Ad-36 presents a conserved alteration of the PI3K/AKT pathway, as mentioned before. *E4orf1* upregulates the expression of glucose transporter 1 (GLUT1) and glucose transporter 4 (GLUT4) in adipocytes through

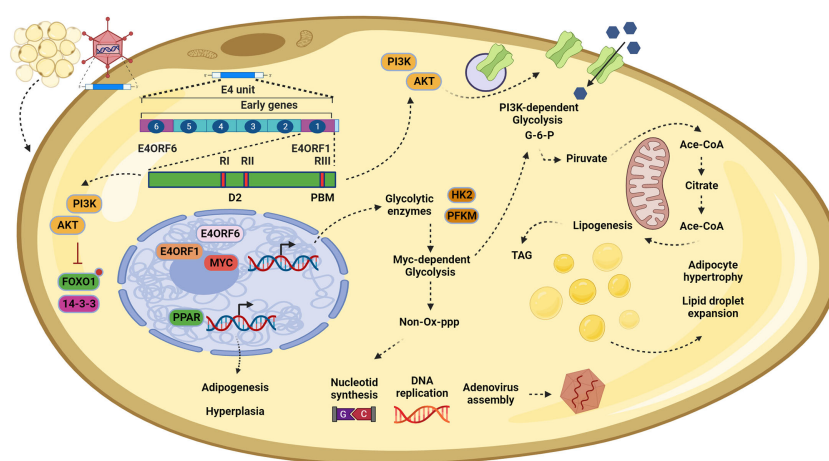


FIGURE 3

In adipocytes, *E4orf1* promotes cell hypertrophy and hyperplasia. Infection by Adenovirus (Ad)-36 in adipocytes also disrupts the PI3K/AKT signaling pathway. Once *E4orf1* is expressed (upper left) it also uses its PDZ domain-binding motif (PBM) to trigger the expression of glucose transporters on the cell membrane, inducing a PI3K-dependent glycolysis (top in the middle). The excess of glucose is converted to glucose-6-phosphate (G-6-P), which enters the mitochondria to synthesize acetyl-CoA and finally triacylglycerols (TAG) that are stored in lipid droplets (in the center). This process promotes lipogenesis in the infected cells and causes lipid droplet expansion, culminating in cellular hypertrophy. Moreover, it is possible that the mechanism described in epithelial cells (MDG) also participates in promoting the hypertrophy mentioned before by generating more G-6-P and creating positive feedback (top in the middle). However, in adipocytes, the MDG may also generate nucleotides via the pentose phosphate pathway (PPP) to sustain viral replication (bottom in the middle). Finally, *E4orf1*, through modulating the PI3K/AKT pathway, regulates the forkhead transcription factor FoxO1, inducing their nuclear exclusion (bottom left). Once FoxO1 is excluded, the brake for the adipocyte differentiation is liberated, allowing the expression of the master regulator for the adipogenesis program *PPARγ*, which in turn upregulates genes involved in commitment to adipocyte cell fate and progress to fat tissue hyperplasia. This figure was created with [BioRender.com](https://www.biorender.com).

the Ras-PI3K pathway. The increase in glucose uptake is dependent on E4orf1–Dlg1 interaction and H-Ras isoform activation (72). In this context of metabolic modulation, the only commonality among the proteins deregulated by E4orf1 is Dlg1. Recently, this protein has emerged as an important mediator of vesicular transport in adipocytes. Through interaction with Sec8 (a protein of the exocyst complex), Dlg1 modulates the anchoring of GLUT4 to the plasma membrane for glucose uptake (73, 74). This deregulation of vesicle trafficking by the viral protein makes sense, as it seems to ensure efficient viral production by promoting constant proliferation through the PI3K/AKT pathway on one hand, and creating positive feedback through glucose uptake on the other (Figure 3). This strategy is also used by other viruses. For example, the E5 oncoprotein of HPV deregulates the endosomal route to recycling EGF receptors to create positive feedback on EGF stimulation (75).

The essence of the oncogenic process lies in chronic and sustained cell proliferation, often accompanied by adjustments in cell metabolism to support the demands of cell growth and division. Certain viruses converge in perturbing the cell metabolism particularly by increasing glycolysis even in the presence of oxygen, a phenomena called the Warburg effect in cancer (39). In cancer cells, such reprogramming is self-defeating because ATP production by glycolysis is very low. However, this problem is overcome by upregulating the GLUT1 to increase glucose import into the cell.

It is worth noting that E4orf1 derived from Ad-36 mirror this metabolic switch in adipose tissue and muscle, and, in fact, utilizes the same mechanism of glucose transporters and oncogenes as cancer cells, such as RAS and MYC (28).

Similar to cancer cells, the benefit for the virus of this metabolic shift is related to the versatility of the glycolysis intermediates, which contribute to the production of nucleotides and amino acids to facilitate viral assembly. E4orf1 derived from Ad-36, by forming a trimer with E4orf6 and MYC, promotes the expression of glycolytic enzymes hexokinase2 and phosphofructokinase 1 to induce PI3K-independent glycolysis (28). This mechanism, in turn, provides intermediaries that enter the pentose phosphate pathway to ultimately provide nucleotides for adenoviral DNA replication (Figure 3). It is important to highlight that we believe that PI3K-dependent glycolysis and MYC-dependent glycolysis are not mutually exclusive. Instead, they both work together in the oncogenic process and the metabolic shift, having an additive effect on cell proliferation or virus replication.

Another interesting correlation is the reliance on glycolysis under hypoxic conditions. It is well documented that the tumor microenvironment operates under fluctuating oxygen gradients, favoring the existence of cell populations that are completely dependent on glucose metabolism, while others do not. This metabolic condition is also shared in adipose tissue of obese individuals, which, in turn, leads to mitochondrial dysfunction and, consequently, low ATP production. This cellular microenvironment is ideal for some Ad members, such as Ad-36, to evolve and modulate key cellular processes through E4orf1 in

favor of viral production. In the context of the oncogenesis, when the virus reaches this cellular microenvironment as a consequence of driving the construction of new virions, some hallmarks of cancer emerge.

Conclusions

Adenoviruses can infect different cells and modulate their physiology to ensure their persistence and viral production. The E4 region encodes the oncoprotein E4orf1, which works to induce optimal viral replication. The E4orf1-induced cell metabolic changes could have the side effect of causing cell transformation. We describe significant changes induced by E4orf1 to modulate signaling pathways and checkpoints of cellular processes, promoting cell cycle entry, avoiding apoptosis, and causing the loss of polarity in epithelial cells; these alterations are considered the hallmarks of cancer. Moreover, E4orf1 promotes increased glycolytic flux in epithelial cells, which, in turn, serves to produce nucleotides for optimal replication. In adipocytes, E4orf1 conserves the disruption of checkpoints and signaling pathways to also induce cell cycle entry, producing hyperplasia and promoting greater glucose uptake, causing hypertrophy. These events are associated with the possible role of E4orf1 in obesity development. Understanding these mechanisms has been essential for describing fine-tuned molecular events in the oncogenic process and has positioned E4orf1 as a potential therapeutic agent for clinical intervention in diabetes.

Author contributions

HA-R and ML-J designed the concept. EF-P, TR, and HA-R completed the final editing of the manuscript. All authors contributed to writing of the manuscript. VC-H, LH-M, and AM-G prepared the figures. All authors have read and approved the manuscript for publication. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors EF-P and ML-J declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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