

The Anti-Tumor Activity of E1A and its Implications in Cancer Therapy

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Received: 29 May 2013 / Accepted: 17 January 2014 / Published online: 7 February 2014
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Abstract The adenovirus type 5 E1A protein (E1A) plays a critical role in anti-cancer gene therapy and has been tested in clinical trials. The expression of E1A significantly reduces tumorigenesis, promotes cell death, and inhibits cancer cell mobility. Chemosensitization is one of the anti-tumor effects of E1A, increasing in vitro and in vivo sensitization of anti-cancer drugs, including cisplatin, gemcitabine, etoposide, doxorubicin, paclitaxel, and tumor necrosis factor-related apoptosis-inducing ligand and histone deacetylase inhibitors in different types of cancer cells. E1A also demonstrates anti-metastasis activity

through various molecular mechanisms such as the repression of protease expression, suppression of HER2/*neu* and downregulation of microRNA (miR-520h). Moreover, E1A has been reported to reprogram transcription in tumor cells and stabilize tumor suppressors such as PP2A/C, p21 and p53. Because E1A plays a potentially significant role in anti-tumor therapy, there exists an urgent need to study the anti-cancer activities of E1A. This paper presents a review of our current understanding of the tumor-suppressive functions and molecular regulation of E1A, as well as the potential clinical applications of E1A.

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Keywords E1A · Gene therapy · Chemoresistance · Metastasis

Introduction

The E1A gene of human adenovirus type 5 is the early gene expressed in an infected cell, providing an appropriate environment for viral replication (Flint and Shenk 1989). Prior to 1991, E1A was usually referred to as an oncogene because of its ability to transform and immortalize rodent cells (Frisch and Mymryk 2002). Later, E1A was found to reverse the transformed phenotype and suppress tumor cell growth in three human cancer cell lines (Frisch 1991) and is therefore now considered a tumor suppressor rather than an oncogene. The gene products of E1A are expressed in two distinctively spliced messenger RNAs (mRNAs), the 13S (289 amino acid) and the 12S (243 amino acid) E1A. Both E1A isoforms can deregulate the cell cycle of the host cells by transcriptional reprogramming and activate the virus gene transcription. These two proteins differ by 46 amino acids encoded in the larger E1A (13S), which is also known as the CR3 domain of E1A. The CR3 domain of

13S E1A is essential for activating virus's early gene expression, however, the 12S E1A's lack of CR3 domain is sufficient to induce transient proliferation state in the host cell and induce transformation (Chinnadurai 2011). The studies characterized in this review included the anti-tumor activities of both 12S and 13S E1A. However, some studies provided different roles of 12S E1A and 13S E1A involvement in distinct anti-cancer activities. In the following section, we will show clearly if the studies use the specific form of E1A. E1A possesses four conserved domains (CR1–CR4) known to contain several protein interaction sequences (Pelka et al. 2008). The CR1 domain of E1A is required for E1A binding with several cellular proteins involved in the chromatin remodeling, including P300/CBP, PCAF, GCN5 and p400 (Chinnadurai 2011); the CR2 domain of E1A is essential for E1A interaction with the tumor suppressor retinoblastoma protein (Rb) and its removal from heterodimeric E2F and DP transcription factor (E2F/DP), and the resultant activation of E2F/DP-dependent transcription (DeCaprio 2009). Both domains (CR1 and CR2) are required for E1A-suppressed tumor growth in melanoma cells, nonetheless, two different mechanisms involve in CR1 and CR2 domains: CR1 domain of E1A is required for its angiogenesis-inhibition, CR2 domain is essential for E1A-induced cell apoptosis (Deng et al. 2002). As a result of preclinical safety and toxicity studies, multiple clinical trials using E1A–liposome complexes have been completed in the past decade (Hortobagyi et al. 2001; Madhusudan et al. 2004; Villaret et al. 2002; Yoo et al. 2001). The intracavity administration of E1A–liposome complexes to patients with advanced breast or ovarian cancer was found to reduce tumor mass and Ki-67 (a cell proliferation marker) expression (Gerdes et al. 1983), as well as to activate apoptosis signals such as tumor necrosis factor- α expression and to induce cell apoptosis (Hortobagyi et al. 2001). E1A combined with paclitaxel treatment has increased paclitaxel-induced apoptosis in ovarian and breast cancer cell models in previous studies (Ueno et al. 2000; Liao et al. 2004). E1A significantly suppresses proliferation and enhances cell death of platinum-resistant ovarian clear cell carcinoma through stabilization of p53 (Itamochi et al. 2007). Several examples of E1A-mediated chemosensitization exist; E1A has greatly increased the sensitivity of SKOV3-ip1 ovarian cancer cells to cytotoxic agents including cisplatin, paclitaxel and doxorubicin by promoting DNA damage and inducing cell death through a p53-independent pathway (Brader et al. 1997). Together with previous safety/toxicity studies (Xing et al. 1997), these results suggest that chemosensitization by E1A may potentially be useful in drug-resistant patients. E1A repressed the transcription of the *HER2/neu* gene causing metastasis inhibition and

enhancing sensitivity to topoisomerase II-targeting anti-cancer drugs, such as etoposide (VP16) and doxorubicin (Adriamycin) (Zhou et al. 2001). E1A has also been shown to mediate tumor suppression by a *HER2/neu*-independent pathway, through several signal transduction pathways, such as enhancing p53-induced apoptosis through p21 and Mdm2 suppression, and activating pro-apoptotic signals by inducing DNA damage response leading to the degradation of Bcl-2 family members and an accumulation of p53 (Yamaguchi et al. 2010; Yamasaki et al. 2012). Phase I/II clinical trials combining E1A and paclitaxel chemotherapy for the treatment of platinum-resistant ovarian cancer have recently been completed. E1A has been shown to inhibit oncogenic signaling pathways, including the inactivation of *HER2/neu*, Akt, and IKK signaling pathways (Liao and Hung 2003; Shao et al. 2001; Yu and Hung 1998), and also activates tumor suppression pathways, resulting in enhanced expression of p53, p21, protein phosphatase 2A/C (PP2A/C), E-cadherin, and FOXO3a (Grootclaes and Frisch 2000; Li et al. 2004; Liao and Hung 2004; Najafi et al. 2003; Su et al. 2011). It deserves mention that 13S E1A but not 12S E1A can transactivate the p21 promoter through Sp1 sites to inhibit tumor cell growth (Najafi et al. 2003). These results suggest that E1A operates more as a tumor suppressor gene than as an oncogene. Our recent study finds that E1A inhibits metastasis in breast cancer cells in *in vitro* and *in vivo* through new epigenetic control of suppressing specific miRNA (miR-520h) (Su et al. 2010). Furthermore, we provide a novel molecular mechanism of E1A-increased paclitaxel sensitization through the stabilization of FOXO3a by the prevention of β -TrCP-mediated ubiquitin-dependent proteolysis through the inhibition of phosphorylation of FOXO3a at Ser-644 by IKK β (Su et al. 2011). Because of the versatility of E1A's anti-tumor activity, it is important to understand the detailed molecular mechanisms associated with E1A-mediated chemosensitization for designing improved clinical trials of combination chemotherapy. Prior studies have demonstrated that E1A has the potential to be a powerful tool against cancer. In this review, we will analyze the current body of research on E1A-induced chemosensitization, metastasis inhibition and other potential therapeutic applications.

E1A Sensitization of Cisplatin Treatment

The anti-tumor activity of cisplatin and other platinum drugs is mediated mainly through the formation of intra-strand DNA cross-links between neighboring purine residues, resulting in the termination of DNA synthesis and the induction of DNA repair or apoptosis (Siddiq

2003). Cisplatin is an important therapeutic tool against several cancers including head and neck, and ovarian and lung cancers. Unfortunately, despite a consistent, initial response leading to therapeutic success, chemoresistance to cisplatin treatment often develops (Galluzzi et al. 2012). The mechanisms involved in cisplatin resistance are associated with amplification of *HER2/neu*, activation of the PI3K/Akt pathway, loss of p53 function, overexpression of Bcl-2 and interference in caspase activation (Siddik 2003). Previous study suggested that 13S E1A induces apoptosis in p53-independent mechanism, whereas 12S product was dependent upon p53 (Teodoro et al. 1995). Samuelson and Lowe (1997) used 12S E1A to demonstrate that 12S E1A causes p53 accumulation and induces apoptosis in Rb-deficient tumor cells. 12S E1A has also been reported to enhance cisplatin sensitization in cancer-resistant cells by blocking the PI3K/Akt pathway (Guinea Viniegra et al. 2002). The CR2 domain of E1A is thought to be required for E1A chemosensitization activity (Deng et al. 2002; Samuelson and Lowe 1997). E1A with a CR2 domain deletion suppresses Bcl-XL deamidation and decreases E1A-induced cisplatin sensitization in SKOV3ip1 ovarian cancer cells (Chang et al. 2006). The expression of p53 is recognized as a necessary process for the induction of several genes associated with cell cycle arrest, DNA repair, and apoptosis, including CDK inhibitor p21, the DNA-damage-inducible Gadd45a gene, and the pro-apoptotic Bax gene (Delmastro et al. 1997). E1A has also been reported to facilitate Mdm4 binding to p53 and inhibit Mdm2 binding to Mdm4, resulting in decreased nuclear export of p53, thereby stabilizing the tumor suppressor p53 (Li et al. 2004). E1A stabilizes p53 within the cell leading to its accumulation and thereby increases chemosensitization. However, it is also evident that E1A can sensitize cells to cisplatin and irradiation, irrespective of the p53 status (Birts et al. 2010). The E1A-interacting protein CtBP was an attractive candidate for several reasons. First, genetic repression of CtBP has been reported to activate several pro-apoptotic genes such as Bax, Noxa, Perp, p21 and Bik in vitro and in vivo. Interestingly, these genes are known p53 targets, CtBP and p53 seem to act through different mechanisms to affect apoptosis signals. Second, the CR4 domain of E1A interacts with CtBP, causing repression of CtBP's activity results in induction of apoptosis signals and decreasing oncogenesis (Chinnadurai 2009). Third, depletion of CtBPs enhances the sensitivity of breast cancer cells and lung cancer cells to chemotherapeutic agents, especially cisplatin and etoposide. Thus, based on the role of CtBP in cisplatin resistance (Grootclaes et al. 2003), we hypothesize that E1A-repressed CtBP may involve in sensitization of cisplatin treatment.

E1A Sensitization of Paclitaxel Treatment

Paclitaxel acts by stabilizing microtubules, obstructing the normal breakdown of microtubules in cell division (Abal et al. 2003). Paclitaxel is a prospective frontline chemotherapeutic agent for the treatment of human breast and ovarian cancers (Bishop and Macarounas-Kirchman 1997). E1A has been shown to sensitize cancer cells to the cytotoxic effects of paclitaxel in in vitro and in vivo in breast and ovarian tumors (Liao et al. 2004; Ueno et al. 2000). Amplification of an important target, *HER2/neu*, has been found in approximately 30 % of human breast and ovarian cancers and is associated with a poor prognosis and worse overall survival for patients (Chen et al. 2003). *HER2/neu* upregulation is often associated with enhanced metastasis and therapeutic resistance to multiple agents such as paclitaxel, cyclophosphamide, methotrexate, 5-fluorouracil, and epirubicin (Chen et al. 2003). Although the function of *HER2/neu* has been extensively studied, the regulatory mechanism and therapeutic-resistance response remain controversial. It has been suggested that E1A downregulates *HER2/neu* through CR1 domain binding to the p300/CBP complex, preventing the interaction between p300/CBP and the *HER2/neu* promoter (Chen and Hung 1997). E1A in combination with paclitaxel causes sensitization in SKOV3-ip1 ovarian cancer cells by inducing apoptosis signals such as Bax, Bad, poly (ADP-ribose) polymerase (PARP) cleavage, and caspase-3 activation, preventing the anti-apoptotic signals Bcl-2 and Bcl-XL and prolonging the survival time of the tumor-bearing animal (Ueno et al. 2000). In breast cancer cells, demonstrating low expression of *HER2/neu*, E1A induced cell death through the induction of poly (ADP-ribose) polymerase (PARP) cleavage in a dose-dependent manner, resulting in paclitaxel sensitization. This resulted in an increase in anti-tumor activity in an orthotopic breast cancer model and prolonged animal survival (Liao et al. 2004). E1A reduced proliferation through the upregulation of phosphoprotein-enriched astrocytes (PEA15) in ovarian cancer cells, promoted the translocation of ERK from the nucleus to cytoplasm, and lead to the inhibition of ERK-dependent transcription and proliferation (Bartholomeusz et al. 2006). FOXO3a belongs to the FOXO family, which activates and/or represses the transcription of genes involved in metabolism, apoptosis, DNA damage repair, and cell-cycle progression (Accili and Arden 2004). FOXO3a has been reported to sensitize paclitaxel to induce apoptosis in *HER2/neu* overexpression cancer cells through increasing Bim (pro-apoptotic BH3-only protein) expression (Sunters et al. 2003). In our previous study (Su et al. 2011), we found that E1A sensitization to paclitaxel in breast cancer cells was accomplished through the

stabilization of FOXO3a, preventing β TrCP-mediated ubiquitin-dependent proteolysis by inhibiting the phosphorylation of FOXO3a at Ser-644 (Hu et al. 2004). E1A induces the expression of PP2A/C, a protein phosphatase involved in multiple cellular functions, including chemosensitization (Liao and Hung 2004), which inhibits transforming growth factor- β -activated kinase 1-activated IKK signaling, thus stabilizing FOXO3a and inducing chemosensitization (Su et al. 2011). Our findings provide a clue to understanding paclitaxel chemosensitization by E1A and offer a mechanistic rationale for utilizing E1A gene therapy as an adjuvant to improve therapeutic outcomes in patients receiving paclitaxel treatment. A previous study indicated that the CR2 domain of E1A is required for the activation of p38 and repression of Akt signaling, which are critical for E1A-induced apoptotic pathway under paclitaxel treatment (Liao and Hung 2003). In addition to discussing an apoptotic pathway to increase paclitaxel chemosensitization, one study suggests that *dl922–947* (the E1A CR2 domain with a 24 bp deletion) induces an abnormal microtubule network and increases microtubule stability. It described a role for microtubules in non-lytic virus exit and demonstrated that paclitaxel synergizes with *dl922–947* by deregulating the activity of mitosis-promoting factors, leading to mitotic slippage, multinucleation and apoptosis induction in ovarian cancer cells (Ingemarsdotter et al. 2010).

E1A Increases the Anti-Tumor Effect of HDAC Inhibitor

Acetylation of histones or genes exerts an important epigenetic control in cellular function and is regulated by histone acetyltransferases and histone deacetyltransferases (HADCs). In cancer cells, the imbalance between histone acetylation and deacetylation generally correlates with tumorigenesis, but the regulatory mechanisms remain unclear (Ko et al. 2008). Chromatin alterations play a fundamental role in cancer onset and progression. Unlike genetic mutations, epigenetic alterations are reversible, and the quest for epigenetic therapies has resulted in the development of several compounds targeting epigenetic enzymes. HADC inhibitor (HDACi) reduces the acetylation status of chromatin and non-histone proteins, resulting in gene inactivation through chromatin remodeling or protein modulation (Lane and Chabner 2009). This has been shown to induce apoptosis, cell cycle arrest and the repression of angiogenesis and metastasis (Ellis and Pili 2010). Over the past several years, many HDACi's and combinations of HDACi's with other anti-tumor agents have been studied in clinical trials, most of which have suggested that HDACi's exhibit convincing

anti-tumor activity (Bolden et al. 2006). Vorinostat (SAHA) was the first HDACi approved by the US Food and Drug Administration for the treatment of cutaneous T cell lymphoma in 2006 (Norris et al. 2009). Clinical trial data have shown that single-agent vorinostat has a limited effect in some types of cancer (Ma et al. 2009). Clinically, the use of HDACi's as monotherapy led to a much more modest anti-tumor activity than the preclinical results suggested, indicating that combination with other agents may be required to achieve relevant clinical effects. E1A did not directly bind to DNA but exerted its chromatin-modifying activity through targeting several cellular proteins, such as the pRb family of pocket proteins, p300/CBP, cyclin/Cdk, CtBP, transcriptional regulator YY1, and the complexes, RACK1 and SWI/SNF (Sang et al. 2002). Reprogramming activity of E1A and the host cell response to this reprogramming lead to transformation, growth arrest or apoptosis. E1A protein binds to Rb family proteins and displaces p300 and CBP from DNA-bound E2F transcription factors, reversing their repression of cell cycle regulator genes (Ferrari et al. 2008). This interaction also causes a significant reduction in global hypoacetylation at H3K18. E1A has been discussed not only for a better understanding of the molecular mechanisms underlying the regulation of transcription, cell division, apoptosis and anti-tumorigenesis, but also of new therapeutics. A recent report suggested that E1A enhanced the pro-apoptotic activity of vorinostat in multiple cancer cells, an effect that was not observed in normal cells (Yamaguchi et al. 2010). The combination of E1A gene therapy with vorinostat showed high therapeutic efficacy with low toxicity in *in vivo* ovarian and breast xenograft models. Vorinostat-enhanced acetylation of histone H3 in the Bim promoter region that upregulates Bim expression, combined with E1A for the therapy of upregulated Egr-1, is directly involved in Bim transactivation in cancer cells. The synergistic effect of E1A on Bim promoter expression drives cancer cells toward apoptosis. This study provided a clear rationale for combined HDACi and E1A gene therapy in future clinical trials.

E1A Increases the Sensitivity of Other Anti-Cancer Drugs

The increased sensitivity of E1A to cytotoxic agents has been reported, despite the distinct mechanisms of action of all of these agents to increase the cell killing ability of E1A (Brader et al. 1997). Etoposide (VP-16) and doxorubicin (Adriamycin) are topoisomerase II-directed anti-cancer drugs, which result in DNA unwinding that causes the DNA strand to break. Previous studies showed that the combination of E1A with these drugs increases cell

apoptosis and chemosensitivity in hepatocellular carcinoma (HCC) and Ewing's sarcoma cells (Li et al. 2001; Zhou et al. 2001, 2005). The anti-tumor efficacy is increased through two molecular mechanisms: first, E1A downregulates the expression of *HER2/neu* and induces apoptotic pathway by increasing PARP cleavage; second, E1A induces the target of etoposide and doxorubicin, topoisomerase II α , and then promotes DNA damage-induced cell apoptosis. Gemcitabine (GEM) is a cytidine analog that has a broad spectrum of anti-tumor activity, blocking in DNA repair and replication; promising results have been indicated in colorectal, breast, ovarian, pancreatic, renal and lung cancer (Silvestris et al. 2004; Westeel et al. 2006; Heinemann 2002; Fleming et al. 2000; Porzner and Seufferlein 2011; Pinilla-Dominguez et al. 2013). E1A sensitized HCC to GEM through the occurrence of apoptosis. E1A enhanced GEM-induced DNA damage by suppression of PARP and NF- κ B signalings in HCC cells (Lee et al. 2003). Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) has been shown to induce apoptosis in a broad range of cancer cell types but not in normal cells, suggesting that it could be a therapeutic agent for cancer (French and Tschopp 1999). TRAIL has demonstrated several anti-cancer effects in in vivo and in vitro, including suppressing tumor growth and improving the survival rate of tumor-bearing mice (Kagawa et al. 2001). Expression of the TRAIL gene in cancer cells induces apoptotic signaling, such as that mediated by caspase 8, caspase 9 and caspase 3, and the combination of TRAIL and chemotherapeutic drugs or ionizing radiation also substantially increases the killing of cancer cells (Gliniak and Le 1999; Chinnaiyan et al. 2000). Researchers are now finding that cancer cell resistance to TRAIL-based therapy may be due to TRAIL not only activating the apoptosis pathway but also activating the tumor survival and proliferation pathway in some cancer cells (Azijli et al. 2013). E1A enhances caspase 8, caspase 9 and Apaf-1 activation, which increases the sensitivity of TRAIL-sensitive (MDA-MB-231) cells and induces the sensitivity of TRAIL-resistant (SKOV3ip1) cells (Shao et al. 2005).

E1A Reduces Cell Mobility and Metastasis

Metastasis is the leading cause of death from primary solid tumors (Nguyen and Massague 2007) and currently has no effective treatment. E1A has been reported to repress various proteases such as type IV collagenase, plasminogen activator, stromelysin, interstitial collagenase and urokinase, all of which are required for tumor cell invasive ability and metastasis (Yu and Hung 1998). Epithelial-

mesenchymal transition (EMT) is an important contributor to the invasion and metastasis of epithelial-derived cancers; the mesenchymal state is associated with the capacity of cells to migrate to distant organs and maintain stemness, allowing their subsequent differentiation into multiple cell types during development and the initiation of metastasis (Thiery et al. 2009). Tumor progression is frequently associated with the downregulation of E-cadherin, which is repressed by several transcription factors, including the Snail, Slug and Zinc-finger E-box binding homeobox (Zeb) families (Nieto 2002; Vandewalle et al. 2009). Interestingly, E1A is known to induce the expression of E-cadherin and to reverse EMT through several molecular mechanisms (Frisch 1994). This phenomenon results from E1A activation of E-cadherin by interrupting the interaction between CtBP and Zeb-1, a negative regulator of E-cadherin (Grooteclaes and Frisch 2000). Patients with cancer that excessively expressed *HER2/neu* have an unfavorable prognosis, a shorter relapse time, metastases and a low survival rate (Chen et al. 2003). Early studies have shown that *HER2/neu* promoter activity can be repressed through Rb interaction with the E1A CR2 domain; the action suppresses the transformed phenotypes and inhibits the metastatic potential of the B104-1-1 cells (Chen et al. 1997). Therefore, E1A has potential in cancer cell gene therapy through targeting *HER2/neu* and metastasis inhibition.

The receptor tyrosine kinase Axl and the ligand growth arrest specific gene-6 have been identified as critical factors in driving tumor cell invasion, migration, proinflammatory cytokine production, antiapoptosis, proliferation and survival (Linger et al. 2008). In ovarian cancer, Axl is a marker of type II (high-grade serous and high-grade endometrioid) ovarian cancer, and genetic inactivation of Axl does not affect tumor cell growth but is sufficient to inhibit the initiation of ovarian metastasis in mouse models (Rankin et al. 2010). In terms of E1A metastasis-suppression competence, Axl can be a potential target. E1A suppresses the signaling pathway of Axl and inhibits downstream molecules that are important for cancer metastasis, including Akt and NF- κ B (Lee et al. 2002). It has been demonstrated that Axl signaling through NF- κ B contributes to cancer cell metastasis and tumor progression (Paccez et al. 2013). Taken together, these results indicate that E1A may also inhibit metastasis through repression of Axl and its downstream molecules, but further investigation is needed to identify the specific effects and mechanisms.

Recent studies have highlighted the importance of miRNA in the regulation of the epithelial phenotype by controlling the EMT regulator (Cano and Nieto 2008). miRNAs are non-coding single strands of ~ 22 nucleotides

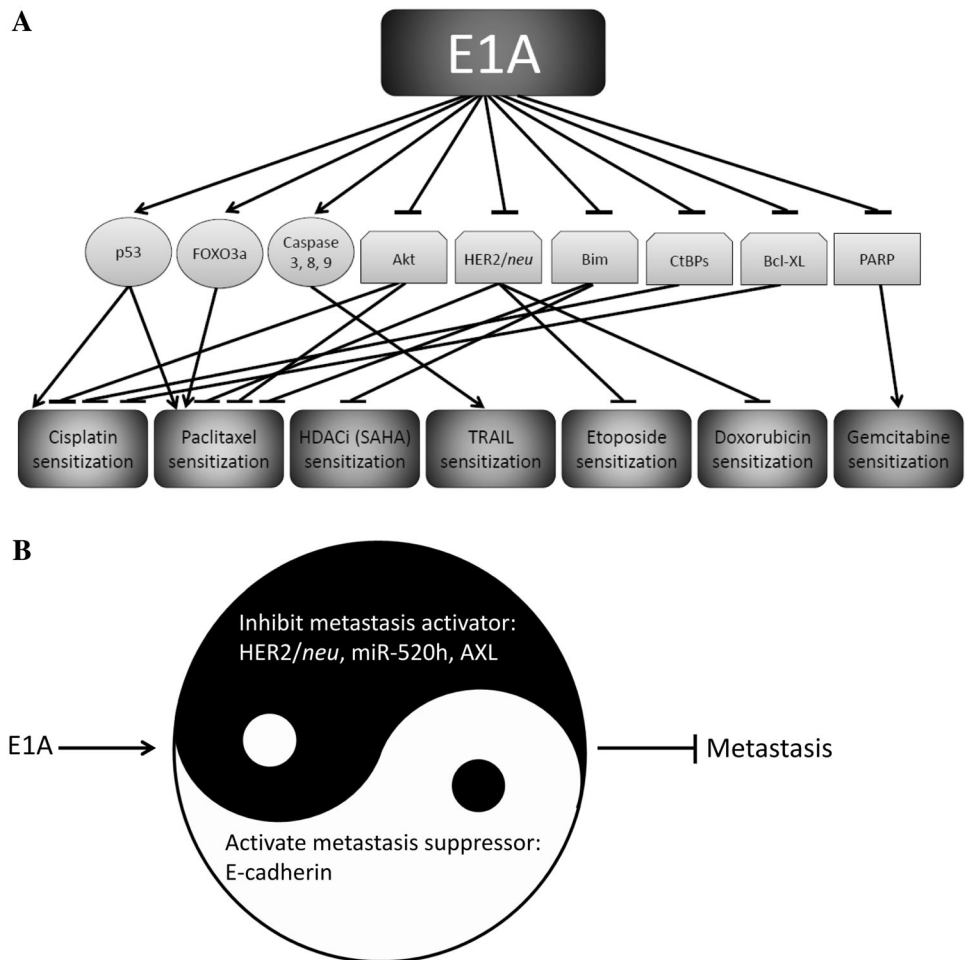
that exert posttranscriptional control on gene expression by sequence-specific interactions with the 3' untranslated region of target mRNAs. Several miRNAs have been reported to be involved in cancer progression, such as the miR-200 family, which controls EMT by targeting Zeb proteins, influencing the metastatic ability of cancer cells (Park et al. 2008). Enforced expression of the miR-200 family prevents lung adenocarcinoma cells from undergoing EMT and from invasion and metastasis (Gibbons et al. 2009). E1A functions in multiple ways to regulate gene expression, including the downregulation of miRNA. Our previous study showed that E1A can mediate metastasis inhibition through the induction of mesenchymal–epithelial transition, by repressing the expression of Twist. This metastasis suppression resulted from the E1A-mediated decrease in miR-520h expression (Su et al. 2010). We propose that E1A repressed miR-520h decreases the migration and invasion ability of MDA-MB-231 cancer cells and decreases tumor growth in an orthotopic xenograft model and lung colonization of metastases in an immunodeficient SCID mice animal models. One target of

miR-520h, the PP2A/C gene, has been shown to be upregulated in E1A-expressing cells. This gene also inhibits IκB kinase activity and downregulates Twist, inhibiting metastasis of cancer cells. We identified this signaling cascade demonstrating that E1A also epigenetically regulates anti-cancer activity through miRNA inhibition.

Concluding Remarks

Researchers are currently working to develop more effective and more specific drugs for cancer treatment but are still hindered by two problems. The first issue is that although treatment may be effective at the primary organ, the cancer cells metastasize, and the disease relapses within a few years. The second issue is that chemoresistance develops in various cancer types, and additional research is necessary to understand how best to resolve this complication. In this review, we referred the current understanding of E1A-mediated anti-cancer activity

Fig. 1 E1A mediates anti-cancer activity and related molecules. **a** E1A synergistic with chemotherapeutic agents and involved molecules. **b** E1A-regulated molecules involves in cell mobility and metastasis of cancer



(Fig. 1). In the last decade, E1A as gene therapy was found to enhance the efficacy of multiple chemotherapeutic drugs through the upregulation of apoptotic molecules such as Bim, Bad, PARP and caspase 3, 8, and 9 in distinct mechanisms. Besides, E1A shows the anti-metastasis activity and transforms tumor cells into epithelial cell types. E1A involves comprehensive tumor-suppressing effects through transcriptional, translational, epigenetic and post-translational regulations on tumor suppressors and oncogenes. Until now, E1A has shown promising therapeutic effects in in vivo and in vitro models. Based on these results, we believe that E1A gene therapy beneficially assists cancer therapy. However, considering the value of its application in cancer treatment, more studies and therapeutic effects should be further provided.

In addition to the well-studied functions of E1A, we searched potential studies involved in E1A-mediated cancer progression. Here, we propose the possibility of anti-cancer functions that may be regulated by E1A (Fig. 2). First, with regard to cancer recurrence, cancer-initiating cells (CICs) are considered to have a central role in tumor initiation and post-treatment relapse. Evidence points to the fact that CICs are particularly resistant to radiotherapy and chemotherapy because of slow turnover and the ability to exclude anti-tumor drugs through the

ABC transporter and the high activity of aldehyde dehydrogenase 1 (ALDH1) (Alison et al. 2012). In breast cancer, the cancer-initiating cell hypothesis is supported by the finding that $CD44^+CD24^{-low}$ cells, frequently found in primary tumors and metastases, exhibit stem cell characteristics, including self-renewal and differentiation along various mammary epithelial lineages (Adams et al. 2008). One attractive study indicates that E1A reduces adhesion molecule CD44 expression through reduced surface HER2/*neu* (Bourguignon et al. 1997). Another study isolated $CD44^+CD24^{-low}$ cells from pleural effusions of breast cancer patients, they provide modification of E1A with cyclooxygenase 2 promoter (Ad5/3-cox2L-d24) and multidrug resistance protein promoter (Ad5/3-mdr-d24) as activating agents, increasing target-specific cell killing in assays (Bauerschmitz et al. 2008). Cox2 and mdr promoters are activating in $CD44^+CD24^{-low}$ breast cancer cells. Their findings may provide support for assessing E1A as an elimination of cancer-initiating cells in breast cancer patients, especially if a modification of E1A efficiency results in an increase in anti-cancer activity. Otherwise, in our preliminary data (unpublished results), we found that E1A in MDA-MB-231 cells significantly reduces the $CD44^+CD24^{-low}$ cell population and the ALDH1-expressing cell population. E1A is likely to be involved in reducing CICs; however, further investigation is needed.

Triple-negative breast cancer (TNBC), a subtype distinguished by undetectable expression of the estrogen receptor, progesterone receptors and HER2/*neu*, represents 15 % of all breast cancers (Amos et al. 2012). Patients with TNBC generally experience a more aggressive clinical course, with an increased risk of disease progression and poorer overall survival. Furthermore, this subtype accounts for a disproportionate percentage of disease-related mortality, in part because of its aggressive natural history and lack of effective targeted agents beyond conventional cytotoxic chemotherapy. Our study presents E1A-enhanced paclitaxel sensitization and metastasis inhibition in MDA-MB-231 cells (TNBC) (Su et al. 2010, 2011), through inhibition of multiple oncogenic pathways. In our unpublished data, E1A inhibited the expression of AXL receptor tyrosine kinase through upregulation of miRNA and sensitized multiple TNBC cell lines to paclitaxel treatment. This suggests that E1A has a substantial capacity to suppress cancer cells, which may overcome the current poor prognosis in TNBC patients.

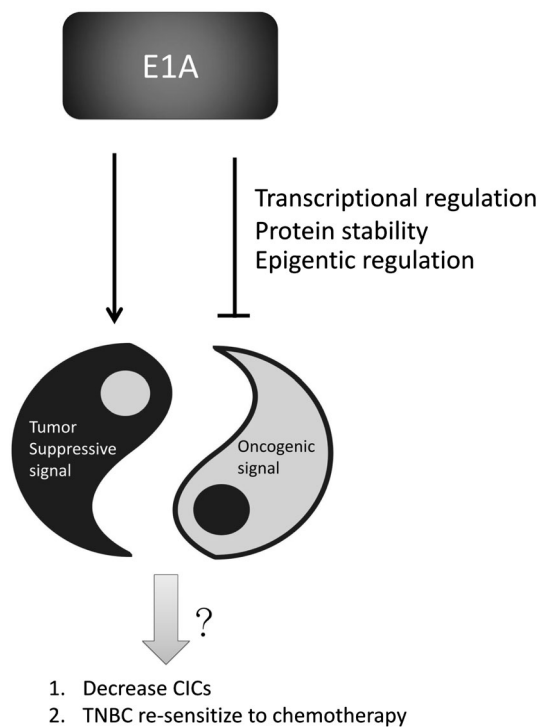


Fig. 2 E1A probably modulates oncogenic signals and tumor-suppressive signals to inhibit cancer-initiating cells (CICs) and sensitizes triple-negative breast cancer (TNBC) cells to chemotherapy

Acknowledgments This work was supported by the National Science Council grant from Taiwan (NSC 102-2314-B-039-200, NSC 102-2314-B-038-028-MY3, NSC 101-2320-B-400-016-MY3); National Health Research Institutes grant from Taiwan (CA-102-PP-41); Ministry of Health and Welfare, Taiwan (DOH 102-TD-C-111-004).

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