E2F-1 cancer gene therapy

Review Article

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Abbreviations: apoptosis protease-activating factor 1, (Apaf-1); human telomerase reverse transcriptase, ((hTERT); multiple drug resistance, (MDR); plaque forming units, (pfu); RNA activated protein kinase, (PKR)

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Summary

Malignant cells frequently have disruption of normal apoptosis pathways due to mutation or dysfunction of apoptosis-related genes. Reconstitution of the apoptotic pathways represents a major strategy for cancer therapy. In this regard, adenoviral vector-mediated p53 cancer gene therapy was first used in human clinical trials. However, the disadvantage of p53 gene therapy is that cancers with a wild-type p53 gene do not respond well to p53 gene therapy, and other mechanisms of p53 resistance are often present in cancer cells. In recent years, E2F-1 has gained increased attention as a target for cancer gene therapy. Transduction of E2F-1 into tumor cells has demonstrated significant anti-tumor effect in vitro and *in vivo*, and in a wide spectrum tumor types regardless of p53, RB, P16 and p73 status. Furthermore, transduction of a very low dose of E2F-1 adenovirus into tumor cells can dramatically sensitize cells to some chemotherapeutic drugs in mouse models of cancer, indicating a new chemosensitization strategy. This may play an important role in chemoresistant tumors such as melanoma and sarcoma. In this review, we shall discuss the function of E2F-1, mechanisms of apoptosis, preclinical studies, as well as the promise and limitations of E2F-1 cancer gene therapy.

I. Introduction

Current cytotoxic chemotherapy for solid tumors is limited by severe adverse events, resulting in a narrow therapeutic index. Gene therapy is an exciting novel approach for treating cancers resistant to currently available modalities. Gene therapy treatment approaches seek to take advantage of molecular differences between normal cells and tumor cells. Several gene therapy strategies have been evaluated, such as (a) correction of defective or aberrant expression of growth control genes, such as p53 or p16; (b) expression of genes that stimulate or augment antitumor immunity, e.g., cytokines; (c) overexpression of a prodrug gene that leads to tumor cytotoxicity, e.g. thymidine kinase; or (d) protection of normal cells from damage by cytotoxic chemotherapeutic agents, e.g., overexpression of the multiple drug resistance (MDR) gene in bone marrow. These approaches have proven efficient in preclinical studies and have now entered clinical trials (Pardoll 1992; Gottesman, et al, 1994; Douglas, 2003; Hughes, et al, 2004).

Apoptosis is recognized as a fundamental mechanism of cell destruction in response to radiation and

chemotherapy. Apoptosis is the highly conserved innate mechanism by which eukaryotic cells commit suicide. This mechanism allows an organism to eliminate unwanted or defective cells by an orderly process of cellular disintegration without inducing an inflammatory response. (Schwartzman & Cidlowski, 1993) Substantial evidence indicates that proliferating somatic tissues exhibit appreciable apoptosis. Malignant cells require antiapoptotic mutations in addition to inappropriate proliferation in order to survive and propagate. (Evan et al, 1995) Studies demonstrated that many apoptosis related genes are mutated and/or dysregulated in human cancers. Induction of apoptosis in malignant cells is a major goal of cancer therapy.

Gene therapy using pro-apoptotic genes that can either trigger apoptosis in chemotherapy-resistant cells, or reconstitute apoptotic pathways to enhance the effect of chemotherapy, is therefore a logical strategy for cancer therapy (Opalka et al, 2002). In that regard, the p53 tumor suppressor gene is the most prominent target for cancer gene therapy. However, the disadvantage of p53 cancer gene therapy is that many cancers with wild-type p53 gene do not respond to p53 gene therapy. The failure of p53 gene therapy is attributed to inactivation of the p53 protein, which can occur by a number of mechanisms (Soddu & Sacchi, 1998; Opalka et al, 2002; Lebedeva et al, 2003). Therefore, gene therapy agents that can induce apoptosis in a p53-independent manner are highly desired. Numerous apoptosis-regulating genes have been evaluated for this purpose, such as p16, p21, p27, E2F genes, FHIT, PTEN and CASPASE genes (Opalka et al, 2002). In this review we shall focus on the potential of E2F-1 as a target gene therapy for cancer.

II. Dural properties of E2F-1: Oncogene and tumor suppressor

The E2F-1 gene is a member of the E2F family of transcription factors. This family composed of six E2F subunits that associated with the DP family members. E2F/DP heterodimers can transactivate or repress gene expression linked with cell-cycle control, apoptosis and senescence (Girling et al, 1993; Dyson, 1998; Phillips and Vousden, 2001). In vivo, E2F transcriptional activity is controlled by interaction with the pocket proteins, the product of the Rb tumor suppressor gene, pRB, and its related proteins p107 and p130. E2Fs are inactivated in early G1 by complex formation with hypophosphorylated pRB. When pRB becomes phosphorylated in mid-late G1 by the actions of cyclin D-dependent kinases, free E2F is released and activated. Based on the interaction with pocket proteins, E2Fs have been categorized into three groups: E2F-1, E2F2 and E2F3 associate exclusively with pRB and are potent transcriptional activators (Dyson, 1998). E2F4, which associates with RB, p107 and p130, and E2F5, which associates with p130, seem to be primarily involved in the active repression of E2Fresponsive genes (Moberg et al, 1996; Takahashi et al, 2000). E2F6 does not interact with pocket proteins and functions as negative regulator of E2F-dependent transcription (Trimarchi et al, 1998). In contrast with other members of the E2F family, E2F-1 is capable of promoting both cell cycle progression and apoptosis (DeGregori et al, 1997; Johnson, 2000).

Oncogenic properties of E2F-1 have been suggested in several studies. Studies carried out in non-malignant cells demonstrate that E2F-1 overexpression in coordination with activated Ras can lead to malignant transformation (Singh et al, 1994; Pierce et al, 1998). Transfer of E2F-1 to quiescent fibroblasts results in entry into the cell cycle and eventually replication of DNA al, 1993). Moreover, (Johnson et transgenic overexpression of E2F-1 caused the formation of skin tumors (Pierce, 1998). The oncogenic properties of E2F-1 are thought to be related to its ability to directly regulate the expression of cell proliferation related genes. To date, several cell-cycle and DNA-replication-related genes have been identified as E2F-1 target genes, such as thymidine kinase, thymidilate synthase, and dihydrofolate reductase (DHFR), which are involved in nucleotide synthesis; Orc1, cdc6 and MCM family proteins which are components of the origin recognition complex; DNA polymerase subunits I and II, and PCNA, which are important for DNA replication; and cell cycle regulators including cyclin E,

cycle A, cdc2, cdk2 and B-myb (Muller & Helin 2000). In addition, a recent study showed that E2F-1 may indirectly enhance proliferation by activating antiapoptotic molecule Bcl-2 (Gomez-Manzano et al, 2001).

However, inconsistent with its role as an oncogene, E2F-1 also has properties as tumor suppressor. Studies in homozygous E2F-1 null (knockout) mice demonstrated increased cell proliferation and neoplasia in several tissues, indicating that E2F-1 also formally functions as a tumor suppressor (Yamasaki et al, 1996; Field et al, 1996; Weinberg, 1996). The identification of human telomerase reverse transcriptase gene ((hTERT) as a target of E2F-1 gives a clue about the molecular basis of E2F-1 tumor suppressor function in vivo (Crowe, 2001). Finally, multiple studies have demonstrated that overexpression of E2F-1 effectively induces apoptotic cell death in a variety of cancers in vitro and in vivo, which does not require functional p53 (Oin et al, 1994; Hiebert et al, 1995; Shan et al, 1996, Hunt et al, 1997; Fueyo et al, 1998; Holmberg et al, 1998; Dong et al, 1999; Liu et al, 1999; Yang et al, 1999, 2000; Atienza et al, 2000). In summary, E2F-1 has two somewhat paradoxical functions: one that regulates cellular proliferation and cycle progression and another that regulates tumor suppression via induction of apoptosis.

III. Mechanism of E2F-1 induced apoptosis

In addition to its established proliferative effect, E2F-1 has also been implicated in the induction of apoptosis through p53-dependent and p53-independent pathways (Phillips and Vousden, 2001; Ginsberg, 2002). Several genes involved in the activation or execution of the apoptotic program have recently been identified as E2F-1 target genes. To date, three pathways of E2F-1 induced apoptosis has been documented (Figure 1), which are (1) a p53-dependent pathway through stabilization of p53 by E2F-1; (2) p53-independent pathways through direct upregulation of apoptotic related genes by E2F-1; and (3) blocking of antiapoptotic pathways through downregulation or inhibition of antiapoptotic molecules.

B. Stabilization of p53 by E2F-1

A role of p53 in E2F-1-induced apoptosis has been demonstrated. In early studies, E2F-1-mediated apoptosis appeared to be p53-dependent, requiring an increase in p53 levels (Xu and Levine, 1994; Hiebert et al, 1995; Kowalik TF et al, 1995). A connection between E2F-1 and p53 was confirmed by the identification of p14^{ARF} as an E2F-1 target gene. E2F-1 can directly transactivate tha p14^{ARF} tumor suppressor gene (p19^{ARF} in rodents) (Bates et al, 1998; Parisi T et al, 2002). ARF can bind and neutralize MDM2, a negative regulator of p53, to prevent p53 degradation (Kamijo et al, 1997; Bates et al, 1998; De Stanchina et al, 1998; Palmero et al, 1998; Stott et al, 1998; Zindy et al, 1998). Thus, transactivation of ARF by E2F-1 leads to p53 stabilization and activation. Studies showed that P14 ARF expression is slightly elevated in Rb -/-cells and p14^{ARF}-deficient cells are partially resistant to



Figure 1. Pathways of E2F-1 induced apoptosis

E2F-1-induced apoptosis (De Stanchina et al, 1998; Zindy et al, 1998). Although ARF contributes to p53 accumulation in response to E2F-1 expression in some circumstances, it has been demonstrated that p14^{ARF} is not necessary for apoptosis to occur. In fact, p14^{ARF} can negatively regulate E2F-1 activity (Mason et al, 2002). The accumulation of p53 by E2F-1 in the absence of p14^{ARF} indicated the presence of additional functional links between E2F-1 and p53 (Russell et al, 2002; Tsai et al, 2002). A recent study showed that E2F-1 can signal p53 phosphorylation in the absence of p19ARF, similar to the observed modifications to p53 in response to DNA damage, which is coincident with p53 accumulation and apoptosis. Additionally, E2F-1-mediated apoptosis is abolished in the presence of caffeine, an inhibitor of phosphatidylinositol 3-kinase-related kinases that phosphorylate p53. These findings suggest that p53 phosphorylation is a key step in E2F-1-mediated apoptosis (Rogoff et al, 2002). Moreover, a recent report demonstrated that ATM is transcriptionally regulated by E2F-1. E2F-1 elevated ATM promoter activity and induced an increase in ATM mRNA and protein levels. This is accompanied by an E2F-1-induced increase in p53 phosphorylation, suggest that ATM serves as a novel, ARF- independent functional link between the RB/E2F pathway and p53 (Berkovich et al, 2003).

Another mechanism of E2F-1 mediated apoptosis that is p53 dependent is related to the binding of cyclin A to the E2F-1 binding site. In a normal functioning cell, cyclin A binds to E2F-1 which then prevents E2F-1 from interacting with p53. Upon cellular DNA damage, cyclin A levels decrease. Thus, less cyclin A binds to E2F-1, allowing for more interaction of E2F-1 with p53. The level of E2F-1-p53 complex increases, eventually leading to p53-dependent apoptosis (Hsieh et al, 2002).

C. Direct transactivation of apoptosis related genes: p73, Apaf-1, caspases 3&7

While much of the research has focused on p53 dependent apoptosis, other studies have shown that E2F-1 can cause apoptosis in the absence of functional p53, suggesting that E2F-1 can induce apoptosis through p53 independent pathways. One clue is provided by the recent finding that E2F-1 can induce upregulation of the p53 homologue, p73 (Irwin et al, 2000). p73 has been shown to carry out similar functions as p53, including transactivation of some of the same target genes and inducing apoptosis or cell cycle arrest. Unlike the mechanism of p53 regulation through p14^{ARF}, E2F-1 can regulate p73 levels directly, through its recognition and transactivation of the p73 promoter (Irwin et al, 2000; Stiewe, 2000; Pediconi et al, 2003).

In addition to p73, recently, upregulation of apoptosis protease-activating factor 1 (Apaf-1) has been implicated in E2F-1-induced apoptosis in melanoma cells. E2F-1 enhanced the expression of Apaf-1 without the cytosolic accumulation of cytochrome c. Apaf-1-deficient melanoma cell lines were resistant to E2F-1, indicating that Apaf-1 is an essential element of E2F-1-mediated apoptosis. Furthermore, Apaf-1 has been identified as a transcriptional target for E2F-1. E2F-1 can directly transactivate Apaf-1, resulting in direct activation of caspase-9 without mitochondrial damage, leading to the initiation of a caspase cascade (Moroni et al, 2001; Furukawa et al, 2002).

Caspase cascades are essential components of the apoptosis machinery. Involvement of caspase cascade in E2F-1 mediated apoptosis has been indicated in previous reports. E2F-1-induced apoptosis was accompanied by caspase-9 activation and inhibited by a specific inhibitor of caspase-9 in K562 sublines overexpressing E2F-1. A recent study has found that enforced expression of E2F-1

results in the accumulation of caspase proenzymes (caspase3 and caspase 7) through a direct transcriptional mechanism (Nahle et al, 2002). Increased caspase levels seem to potentiate cell death in the presence of p53-generated signals that trigger caspase activation. (Muller et al, 2001; Ma et al, 2002; Nahle et al, 2002)

Besides the genes above which have been identified as E2F-1 target genes, other apoptosis- related genes may play a role in E2F-1-induced apoptosis. Double-stranded RNA activated protein kinase (PKR) and mitochondrial apoptosis-inducing factor have been implicated in the E2F-1-induced apoptosis pathway in colon cancer (Vorburger et al, 2002, 2003), and interestingly, a linkage between ASK1 and PKR was identified recently (Takizawa et al, 2002). DNA microarray analysis demonstrated that ectopic expression of E2F-1 can upregulate expression of pro-apoptotic genes of bcl-2 family (Ma et al, 2002; Stanelle et al, 2002) and overexpression of E2F-1 by adenovirus-mediated gene transfer upregulated ASK-1-apoptosis signal- regulating kinase 1 in melanoma cell lines (McMasters, unpublished data). However, whether these genes are regulated by E2F-1 at transcription level and their importance in E2F-1induced apoptosis remains to be further elucidated.

D. Inhibition of antiapoptotic pathways-NF-kappaB and Mcl-1

Activation of pro-apoptotic signals, p14ARF, ATM, P73 and Apaf-1 depends on the transcriptional activity of E2F-1. However, solid evidence indicates the transactivation domain of E2F-1 is not necessary for E2F-1-mediated apoptosis (Hsieh et al, 1997; Phillips et al, 1997), suggesting that it may be mediated through alleviation of E2F-dependent transcriptional repression. Studies have shown that E2F-1 can induce apoptosis via a death receptor mechanism by inhibiting activation of NF B. By downregulating TRAF2 protein levels, TNFreceptor-mediated NF B and JNK activation was impaired. E2F-1 overexpression was also found to inhibit NF B activity in a cell cycle dependent manner by using E2F-1 (+/+) and E2F-1 (-/-) murine embryonic fibroblasts (Tanaka et al, 2002). These results further support that E2F-1 promotes apoptosis by blocking or inhibiting antiapoptotic signals, namely NF B activity. Additionally, downregulation of Mcl-1 level, an anti-apoptotic Bcl-2 family member was noted in E2F-1-overexpressing cells, suggesting that transcriptional repression of antiapototic molecules may be an alternative mechanism of E2F-1induced apoptosis (Dong et al, 1999; Yang et al, 2000; Elliott et al, 2001). This was further supported by recent study which showed that E2F-1 potently represses the expression of Mcl-1 by directly binding to the Mcl-1 promoter. This transcriptional repression is direct and dependent upon E2F-1's DNA-binding domain, but does not require the transactivation domain of E2F-1. Furthermore, cell lines constitutively expressing Mcl-1 are resistant to E2F-1-mediated apoptosis--suggesting that Mcl-1 downregulation is a necessary event in the p53independent apoptotic process (Croxton et al, 2002). Represses transcription of the hTERT gene by E2F-1 may

also contribute to E2F-1 induced apoptosis (Crowe et al, 2001).

IV. E2F-1 as potential target for cancer gene therapy: Preclinical studies A. *In vitro* and *in vivo* anti-tumor effect by adenovirus E2F-1 gene therapy

It has previously been shown that transient transfection of E2F-1 plamid into quiescent rat embryo fibroblasts induced apoptosis, indicating the therapeutic potential of this gene. A recombinant adenovirus vector containing the transgene E2F-1 under control of the cytomegalovirus promoter was constructed by Dr. Liu (Liu et al, 1999). To date, anti-tumor effect of E2F-1 gene therapy has been evaluated in a wide variety of malignant cells in vitro and in vivo by adenovirus-mediated E2F-1 gene transfer. The first in vitro gene therapy study with E2F-1 adenovirus was performed in human breast and ovarian carcinoma cell lines. Apoptotic cell death occurred in four of the five cell lines within 48 h of transduction with the E2F-1 adenovirus and the induction of E2F-1mediated apoptosis did not require wild-type p53 (Hunt et al, 1997). Later, Fueyo et. al evaluated the anti-tumor effect of E2F-1 adenovirus in human glioma in vitro and in vivo. This study showed that the adenovirus-mediated transfer of exogenous E2F-1 protein precipitated generalized apoptosis in gliomas. The treatment with E2F-1 adenovirus of nude mice carrying subcutaneous gliomas arrested tumor growth (Fueyo et al, 1998). The effect of E2F-1 gene therapy on head and neck squamous cell carcinoma was evaluated by Liu et al Two cell lines, Tu-138 and Tu-167, which harbor p53 mutations but express different levels of the retinoblastoma protein, were used in this study. Overexpression of E2F-1 via an adenoviral vector suppressed in vitro and *in vivo* growth of head and neck squamous carcinoma cell lines through induction of apoptosis (Liu et al, 1999).

The possible therapeutic benefit of E2F-1 gene therapy in sarcomas has been evaluated as well. Yang et.al study showed that E2F-1 overexpression by adenovirusmediated gene transfer in MDM2-overexpressing sarcomas resulted in marked growth inhibition and rapid loss of cell viability which was due to induction of apoptotic cell death. E2F-1 overexpression was associated with a marked decrease in MDM2 levels and caspase 3. Because MDM2-overexpressing tumors are often resistant to p53 gene therapy, adenovirus-mediated E2F-1 gene therapy may be a promising alternative strategy for the MDM2 overexpressing tumors (Yang et al, 1999). Furthermore, a recent report from Vorburger's group showed that transducing E2F-1 gene into SKLMS-1, a human leiomyosarcoma cell line which has a p53 point mutation resulted in increased entry into S-phase despite upregulation of the p21 protein, followed by apoptosis. In vitro single-dose administration of 2000 viral particles (vp) Ad5E2F1 per cell showed significant growth inhibition of the SKLMS-1 cells compared to controls. Treatment of SKLMS-1 tumor bearing BALB/c nu/nu mice with 13 consecutive intratumoral injections of AdE2F at a dose of $2x10^9$ plaque forming units (pfu) resulted in significant growth inhibition of the sarcoma. Complete tumor regression was seen in 2 out of 7 mice. These findings demonstrate that adenovirus-mediated overexpression of E2F-1 results in significant growth inhibition of leiomyosarcoma in vitro and *in vivo* (Vorburger et al, 2002).

The effect of adenovirus-mediated E2F-1 gene transfer on human melanoma cell growth was investigated by Dong et al (Dong et al, 1999). E2F-1 overexpression resulted in growth inhibition by G2 arrest, followed by apoptotic cell death. Adenovirus-mediated E2F-1 gene transfer efficiently induced widespread apoptosis in human melanoma cell lines containing wild-type and mutant p53, suggesting that E2F-1 cancer gene therapy is effective regardless p53 status. Anti-apoptotic proteins of the Bcl-2 family, notably Mcl-1 and Bcl-XL, may be involved in mediating the response to E2F-1. These data suggest that adenovirus-mediated E2F-1 gene therapy may be effective in the treatment of melanoma (Dong et al, 1999). Similar studies have been performed in other cancer cell lines such as esophageal carcinoma (Yang et al, 2000), pancreatic carcinoma (Elliott et al, 2002), colon cancer (Elliott et al, 2002), gastric carcinoma (Atienza et al, 2000), and nonsmall-cell lung cancer (Kuhn et al, 2002). A universal anti-tumor effect by adenovirusmediated E2F-1 gene transfer has been observed regardless tumor type and genetic background (Figure 2).

B. Adenovirus-mediated E2F-1 gene therapy sensitize tumors to chemo/radio therapy

Traditional chemotherapy have had limited success in treating several types of cancer such as sarcoma and metastatic melanoma. Though the mechanisms of chemoresistance remain ambiguous, the defect in the normal apoptosis machinery is likely an important contributor to chemoresistance (Lutzker and Levine, 1996). Studies have suggested that the induction of E2F-1 following chemotherapy endogenous may correlated with chemosensitivity to some of chemotherapeutic agents, and that E2F-1 may play a role following in mediating apoptosis exposure to chemotherapeutic agents (Nip et al, 1997; Banerjee et al, 1998; Meng et al, 1999).

Melanoma cells are resistant to most common chemotherapeutic agents, alone and combination treatment. A recent study demonstrated that transduction of the E2F-1 gene in melanoma cells markedly increased cell sensitivity to some chemotherapeutic agents, especially to topoisomerase II inhibitors such as Adriamycin and etoposide, thereby producing a synergistic effect on apoptotic cell death. Moreover, topoisomerase II inhibitors also cooperated with Ad-E2F-1 to enhance antitumor effect in an *in vivo* nude mice model. When combined with Adriamycin or etoposide, E2F-1 adenovirus therapy resulted in approximately 87% and 91% decrease in tumor size, respectively, as compared to



Figure 2. In vitro anti-tumor effect by adenovirus E2F-1 gene therapy. **a,b,c**: In vitro growth inhibition by adenovirus E2F-1 gene therapy. **d**: Loss in cell viability after infection with adenovirus E2F-1. **e**: E2F-1 overexpression caused G2 arrest, followed by apoptosis. **f**: PARP cleavage assay confirmed apoptotic cell death.



Figure 3. Adenovirus-mediated E2F-1 gene therapy sensitize melanoma to Etopside in vitro.

A: The effect of E2F-1 expression on the sensitivity of melanoma cell lines to Etoposide. B: The synergistic effect of combination treatment with Ad-E2F-1 and toposoimerase II inhibitors on human melanoma cell death. Etoposide (Etop), Adriamycin (Adr). C: PARP cleavage assay demonstrated that overexpression of E2F-1 sensitize melanoma cells to toposoimerase II inhibitors by induction of apoptosis.



Figure 4. *In vivo* anti-tumor effect by combination treatment with E2F-1 adenovirus and chemotherapeutic agents. **A and B**: In vivo antitumor effect of the combination treatment of adenovirus-E2F-1 and Etopside in melanoma mouse model. **C and D**: In vivo antitumor effect of the combination treatment of adenovirus-E2F-1 and Camptothecin in colon cancer mouse model.

controls (P<0.002), while a decrease in tumor size of around 37% was observed by adriamycin or etoposide treatment alone (**Figure 3 and Figure 4**). These results suggest a new chemosensitization strategy for melanoma gene therapy (Dong et al, 2002). Similar studies have been carried out in MDM2 overexpressed osteosarcoma cells *in vitro* and *in vivo*, in which an additive antitumor effect by

combination treatment with E2F-1 adenovirus and topoisomerase II inhibitors was observed (Yang et al, 2001). Enhanced chemosensitivity to camptothecin by E2F-1 was also reported in colon cancer mice model (Dong et al, 2003; **Figure 4**). However, E2F-1 seems to have no effect on chemosensitivity to Taxol, cisplatin and 5-fluorouracil (Dong et al, 2002).

Human gioma is another chemoresistant malignancy. Less than 30% of patients with glioblastoma multiforme respond to adjuvant chemotherapy. Overexpression of E2F-1 by adenovirus-mediated gene transfer sensitized malignant human gioma cell lines to BCNU and temozolomide (Gomez-Manzano et al, 2001). Thus, E2F-1 may lower the therapeutic dose threshold for conventional chemotherapy agents.

In addition, E2F-1 also has properties as a radiosensitizer. Expression of E2F-1 in p53-/fibrosarcoma cells enhanced the cytotoxic effect of ionizing radiation *in vitro* and *in vivo* in a mouse tumor model. These results suggest that E2F-1-dependent activation of an S-phase checkpoint is p53 independent and that E2F-1 possesses radiosensitizing properties in the absence of p53 (Pruschy et al, 1999).

V. E2F-1 cancer gene therapy: perspectives and limitations

The therapeutic index of currently available modalities for most metastatic and locally advanced malignancies is low. The disruption of normal apoptotic pathways in malignant cells is a critical factor for chemoor radio-resistance. Therefore, a cancer gene therapy strategy has developed to replace or reconstitute the defective apoptotic pathways in malignant cells, thereby restoring the apoptosis or sensitizing cells to chemo/radiation induced apoptosis. Dysegulation of E2F transcription factors, via alterations in the p16-cyclin D-Rb pathway, is a key event in the malignant progression of most human malignancy. Overexpression of E2F-1 by adenovirus-mediated gene transfer has widespread killing effect on tumor cells and is independent p53, RB, p73 and Arf tumor suppressor genes. Unlike p53 gene therapy, which only works in p53 mutant cancers, E2F-1 cancer gene therapy may benefit a wider spectrum of cancer patients regardless of genetic background. However, due to the oncogenic ability of E2F-1, the safety of E2F-1 cancer gene therapy may be a concern in clinical utility. New results indicated that E2F-1 also involved in apoptosis suppression under some circumstances (La Thangue, 2003; Wikonkal et al, 2003), which raised more questions on its suitability as a gene therapy agent. Studies on the molecular basis of E2F-1-induced apoptosis demonstrated that the transactivation and apoptosis functions of E2F-1 are separable. A carboxy-terminal deletion mutant of E2F-1 can induce apoptosis without stimulating DNA synthesis (Hsieh et al, 1997; Phillips et al, 1997). Therefore, development of a new E2F-1 adenovirus vector that still maintains its apoptotic function but no longer possesses oncogenic ability may improve its potential clinical utility.

Another concern for E2F-1 cancer gene therapy is the toxicity to normal cells, although cancer cells appear to be much more sensitive to adenovirus-mediated E2F-1 gene transfer than normal fibroblasts (McMasters, unpublished data). Development of new vectors which selectively target tumors using tissue-specific or tumor-specific promoters may be a promising approach for E2F-1 cancer gene therapy.

Initial clinical trials should choose models that can effectively and safely evaluate the potential of E2F-1 gene therapy. Direct intratumoral injection of E2F-1 adenovirus may allow the maximum doses of E2F-1 into the tumor while minimize the side effects to normal cells. From this standpoint, melanoma is an ideal disease in which to test gene therapy clinically, because cutaneous in transit disease is amenable to local intratumoral injection or isolated limb perfusion. Colorectal cancer metastatic to the liver may be treated by hepatic artery delivery of gene therapy, or in some cases by direct intratumoral injection, although hepatic toxicity is an important consideration. Therefore, we believe that it is not beyond reason that adenoviral vector E2F-1 gene therapy could be applied clinically in such human cases of melanoma or colon cancer. Moreover, the ability of E2F-1 to simultaneously stimulate pro-apoptotic signals while inhibiting antiapoptotic ones makes it a primary candidate for combination "chemogene" therapy. Transduction of very low dose of E2F-1 adenovirus into tumor cells can dramatically sensitize cells to some chemotherapeutic drugs in mice models for cancer, indicating a new chemosensitization strategy for chemoresistant malignancy such as melanoma, glioma and sarcoma.

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