E2F1 death pathways as targets for cancer therapy

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Abstract

Defects in apoptotic programs contribute to a number of human diseases, ranging from neurodegenerative disorders to malignancy, and treatment failure. The genetic basis for apoptosis implies that cell death can be disrupted by mutations, raising the intriguing possibility that cell numbers can be regulated by factors that influence cell survival. It is well documented that the E2F1 transcription factor is a key regulator of apoptotic programs. E2F1-induced cell death occurs via multiple pathways, some of which involve the tumour suppressor p53, and autonomous of p53. This has led to the opinion that E2F1 functions as a tumour surveillance factor, detecting aberrant proliferation and engaging apoptotic pathways to protect the organism from developing tumours. Frequently, novel players are discovered that expand the interpretation of apoptosis control by E2F1. This information will help to produce new strategies to exploit E2F1-induced apoptosis for therapeutic benefit.

Keywords: E2F1 • apoptosis • signalling pathways • cancer • molecular therapy

Introduction

The E2F transcription factor is best known for its ability to regulate cell cycle progression by coordinating a large group of genes involved in G1-to-S phase transition. E2F regulated genes include cell cycle regulators, such as Cyclin E and Cdc25A, and genes that encode essential components of the DNA replication machinery [1]. In agreement with the idea that E2F is an important regulator of cell proliferation, studies in several experimental systems have shown that the ectopic expression of E2F is sufficient to drive quiescent cells into S phase, while the inhibition of E2F-dependent transcription blocks cell proliferation [2–4]. E2F cell cycle activity is controlled through the temporally regulated physical association of pocket proteins. The hypophosphorylated retinoblastoma protein pRB represses E2F activity by shielding the E2F transactivation domain leading to restriction of cell cycle progression [5]. During the course of cell cycle progression through the restriction point, pRB becomes phosphorylated by the activity of cyclin-dependent kinases (Cdks) resulting in the release of E2F, thereby causing its activation (Fig. 1). It is currently believed that most, and perhaps all, human cancers contain mutations that comprise pRB function...
and that this contributes to their ability to proliferate in an environment that would cause normal cells to arrest. E2F is a family composed of at least eight members that can be divided into distinct subgroups on the basis of their structural and functional similarities (reviewed in ref. [6]). E2F1, E2F2 and E2F3a are transcriptional activators, whereas E2F3b, E2F4, E2F5, E2F6 and E2F7 act as repressors of transcription. The most recently identified E2F family member, E2F8, resembles the organization of E2F7 in the presence of two separate DNA-binding domains. Like E2F7, E2F8 can repress transcription and delay cell cycle progression. All E2F proteins except E2F7 and E2F8 function in heterodimeric complexes with DP family (DP1-DP4) proteins. From mutant mouse models it became clear that different E2F and DP subunits perform distinct, perhaps overlapping functions in the control of cell cycle progression [7], as well as unique roles during development, tissue homeostasis and apoptosis [8]. For example, E2F3 is important for the control of cellular proliferation, while E2F4 and E2F5 are essential for G1 control and cell cycle exit as well as for normal mouse development. Previous studies suggest a role for E2F7 in facilitating cell cycle arrest in both G1 and G2 phases [9]. E2F1, E2F2 and E2F3 exclusively bind pRB. In contrast, E2F4 can associate with all pocket proteins, while E2F5 solely binds p130. E2F6 as well as the newest family members E2F7 and E2F8 lack a transactivation domain and do not interact with any pocket protein.

Recent genomic studies have found that the function of the E2F transcriptional program extends further than the G1/S transition. Chromatin immunoprecipitation experiments [10–13], expression profiling studies [14–17] and a recently performed proteomic analysis [18] showed that E2F proteins bind to and modulate expression of a large fraction of genes with diverse functions, suggesting a widespread role for E2F in the human genome. Currently, the best-characterized activity, especially at the hands of E2F1, in addition to its traditional role in cell cycle progression is its ability to induce apoptosis [19–22]. In some set-
ting, another member of the E2F family, E2F3, also induces apoptosis. However, this E2F3-induced apoptosis was found to be E2F1-dependent and is largely attributed to the ability of E2F3 to transactivate the E2F1 gene, indicating that accumulation of crucial levels of E2F1 activity, and not total E2F activity, is essential for the induction of apoptosis [23].

**Molecular mechanisms of E2F1-induced apoptosis**

A large number of studies clearly support the role of E2F1 as a tumour suppressor rather than an oncogene [24–26]. Under deregulated conditions the activity of E2F1 is linked to events that determine cell fate through the induction of apoptosis, thus protecting the organism against oncogenic transformation. Mice, for example, lacking E2F1 (E2F1−/−) exhibit defects in apoptosis together with an increased incidence of tumour development [24, 25], and the level of apoptosis seen in RB−/− mice is suppressed by E2F1 deficiency [26].

Different mechanisms have been attributed to the ability of E2F1 to cause apoptosis, which can occur through both p53-dependent and independent pathways (Fig. 2). In the p53-dependent pathway, p53 accumulates following E2F1 expression [27] through activation of the CDKN2A transcript p14ARF, which in turn interacts with Mdm2, thereby preventing Mdm2 from targeting p53 for ubiquitination and subsequent degradation [28]. Lately, additional ARF-independent pathways have been described [29], and some reports even imply a negative feedback by ARF since over-expression of ARF inhibits E2F1-dependent apoptosis, and targets E2F1 for proteasomal degradation [30]. E2F1-induced apoptosis in the absence of ARF was shown to correlate with p53 phosphorylation at residues that are also phosphorylated in response to DNA damage [29, 31]. Moreover, induction of both apoptosis and p53 phosphorylation by E2F1 are abolished by the ATM and ATR protein kinase inhibitor caffeine [31], supporting that E2F1 uses the ATM signalling pathway to induce p53 and Chk2 phosphorylation and thereby apoptosis [32, 33]. Correspondingly, it was reported that the E2F1 transcription factor elevates ATM promoter activity by direct binding, which is accompanied by enhanced p53 phosphorylation [34]. In addition, E2F1 interacts directly with p53 via the cyclin A–binding domain of E2F1, and this interaction enhances p53 apoptotic activity in response to DNA damage [35].

E2F1-induced apoptosis occurs also independent of p53 in tissue culture and transgenic mice [36–38], and pRB has been shown to protect p53-null cells from apoptosis in an E2F1-binding–dependent manner [39]. Apoptotic targets of E2F1 in the absence of p53 include the p53 homolog protein p73 [40, 41] and apoptosis protease-activating factor 1 (Apaf1) [42] both of which are transcriptionally regulated by E2F1. This initiates the assembly of Apaf1 with cytochrome c followed by procaspase-9 activation and successive initiation of proapoptotic effector caspases including Caspase-3, -6 and -7 [42].

Mapping studies revealed that the apoptotic ability of E2F1 requires the DNA-binding domain but not its transactivation function, since mutants of E2F1 that lack the transactivation domain are still able to induce cell death [36, 37, 43]. From these experiments, it was supposed that proapoptotic E2F1 target genes are activated by removal of E2F1/RB repression rather than direct transactivation. Moreover, Bell et al. [44] recently reported DNA-binding–independent cell death from a minimal proapoptotic region of E2F1 that is consequently unable to transactivate, repress or derepress E2F target genes. However, since this activity is also present in E2F2 and E2F3 proteins, it might therefore define a distinct mechanism of death by E2F proteins.

Because normal cells proliferate without suffering E2F1-induced apoptosis, its proapoptotic potential is evidently held in check during development. How the switch between these activities is controlled is not well understood. Integration of external signals appears to play an important role in determining the sensitivity to E2F1-induced apoptosis. For example, cell survival signals via the PI3/AKT and the EGFR/Ras/Raf pathway have been shown to promote E2F1-driven cell proliferation by suppressing E2F1-induced apoptosis [45–47]. In addition, DNA damage signals have been suggested to specifically activate E2F1-dependent transcription of proapoptotic genes [48–50]. These changes stabilize E2F1, increase its transactivation potential and allow it to preferentially bind the promoters of some proapoptotic genes. Overall, the final decision of whether E2F1 leads to cell proliferation or apoptosis might thus depend on the genetic status or molecular background of the cell. This means that E2F1 would func-
tion strictly as a growth promoter if pathways that mediate E2F1-induced apoptosis are disabled.

**E2F1 sensitizes tumour cells to DNA damage**

DNA-damaging agents are regularly used to fight various human cancers. Many of the drugs cause DNA damage and the inflicted DNA damage activates apoptotic programs that lead to cell death. Forced expression of E2F1 has been shown to sensitize tumour cells to the proapoptotic signals generated by chemotherapeutic drugs or ionizing radiation [51–56]. In addition, endogenous E2F1 is up-regulated after DNA damage in a manner analogous to that of p53, suggesting its direct role in responsiveness to conventional genotoxic stress signals [57]. In response to DNA damage, the E2F1 protein is stabilized through distinct mechanisms, including direct phosphorylation by the ataxia-telangiectasia mutated (ATM) kinase, the ATM and RAD3-related (ATR)
kinase and the Chk2 kinase [32, 48, 58], and also by acetylation through p300/CREB-binding protein-associated factor (P/CAF) [50]. Activation of the ATM DNA damage response pathway is commonly observed in a variety of early-stage tumours, suggesting that this checkpoint response functions to suppress the development of cancer. Moon et al. [59] has demonstrated that E2F1 is a critical determinant of the cellular response in cancer cells to genotoxic stress. Taking advantage of Drosophila dDP and de2f1;de2f2 mutant animals, it was shown that the role of de2f1 within individual developing wing discs exposed to γ-irradiation is completely context-dependent. dE2F1/dDP appears to protect non-proliferating cells and sensitizes proliferating cells to γ-irradiation-induced apoptosis. The loss of the pRB-dependent cell cycle checkpoint might allow cancer cells to enter S phase and initiate apoptosis under conditions where normal cells would undergo a G1 arrest and initiate DNA repair. In addition, E2F can increase the effectiveness of chemotherapeutic agents that are most active in S phase cells and/or that require the presence of a particular cell cycle–dependent target for the induction of cell death [60, 61]. This connection between DNA damage and E2F1-dependent apoptosis is of particular significance. The frequent deregulation of E2F1 in human tumours, taken together with its apoptotic potential and its stabilization after damage suggest that E2F1 may play an important role in the enhanced sensitivity of tumour cells to DNA damage-induced cell death. In agreement with this, increased levels of proapoptotic target genes of E2F1 such as adenoviral E1A and p73 have been invoked as a potential basis for selective killing of cancer cells, including p53-defective tumour cells, by DNA-damaging agents relative to normal cells [62–64].

E2F1 guided death pathways in cancer

In the past few years, a large number of novel E2F1-regulated target genes or gene networks functioning in the apoptotic program have been identified. From these studies it becomes evidently clear that E2F1 is a key regulator of the apoptotic machinery by being involved in many aspects of programmed cell death, both by activating proapoptotic genes and through the inhibition of antiapoptotic survival signals.

Activation of proapoptotic genes

E2F1 induces the expression of the proapoptotic Bcl-2 homology 3 (BH3)-only proteins PUMA, Noxa, Bim, Hrk/DP5 and Bik in cancer cells through a direct transcriptional mechanism [65, 66]. In fact, E2F1 binds to the promoters of these genes and transactivates them by a p53-independent mechanism. BH3-only proteins are members of the Bcl-2 protein family and are required for the execution of apoptotic cell death by integrating diverse apoptotic stimuli into a common death pathway governed by other multidomain Bcl-2 family members. While some BH3-only proteins, such as Bid, may chaperone the activation of Bax and Bak at the mitochondrial membrane [67], most others antagonize the functions of the anti-apoptotic Bcl-2 family members [68]. Reversely, reducing the expression of Noxa and PUMA by RNA interference diminished apoptosis induced by E2F1 [65]. Like E2F1 itself, some of these proteins, for example, Bik and PUMA are also up-regulated in response to chemotherapeutic agents [65, 66]. Consistent with their responsiveness to E2F1, it has been shown that the DNA damage-induced elevation of PUMA and Bik is abolished by suppression of E2F1 activity, indicating that both pathways contribute to the apoptotic response to damaging agents. Increased expression of proapoptotic Bcl-2 genes including Bad, Bak and Bid after E2F1 activation in p53-deficient tumour cells was also reported in a gene array analysis by Stanelle et al. [14]. In the same study, KIAA0767, termed D(eath)-(inducing)-P(rotein), was identified as a relevant apoptotic target of E2F1. The DIP protein is localized in the mitochondria and mediates E2F1-induced apoptosis independently of p53 in a partially caspase-independent manner [69]. In addition, transcriptional activation of the second mitochondrial activator of caspases or direct inhibitor-of-apoptosis-binding protein with low pl (Smac/DIABLO) was reported as a mechanism by which E2F1 promotes p53-independent apoptosis via the mitochondria [70].

Caspases are essential components of the apoptotic machinery. These proteases are synthesized as inactive proenzymes and processed to an active state during apoptotic cell death. Initiator caspases
(Caspase-2, -8 and -9) trigger a cascade that results in the activation of effector caspases (Caspase-3 and -7), which in turn produce the characteristic morphological changes associated with apoptosis. Enforced E2F1 expression has been shown to result in the accumulation of Caspase-2, -3, -7, -8 and -9 through a direct transcriptional mechanism [71]. E2F1 can facilitate caspase activation through p53-dependent signals, resulting in mitochondrial release of cytochrome c, while simultaneously increasing caspase expression through direct caspase promoter binding that is independent of p53. By up-regulation of caspases, particularly Caspase-8, E2F1 might sensitize cells to death-inducing ligands such as tumour necrosis factor (TNF).

Another novel death effector protein of E2F1 is SIVA, which was identified to be directly up-regulated by E2F1 and p53 via their corresponding consensus sites in the SIVA promoter [72]. SIVA is a proapoptotic protein containing a death domain that is expressed on a broad scope of tissues, including cells of the immune system and the CNS as well as tumour cells. As a potential mechanism for apoptosis induction by SIVA, its interaction with members of the TNF-receptor family and antiapoptotic Bcl-2 family members has been considered [73]. These studies suggest that SIVA may function through multiple mechanisms possibly dependent on the cell type and apoptotic stimulus. In addition to the findings by Fortin et al. [72] indicating that the SIVA gene exhibits a striking induction during p53-mediated neuronal apoptosis, up-regulation of SIVA was also observed in p53-deficient hepatoma cells in response to cisplatinum-induced apoptosis [74], suggesting that it is also regulated in a p53-independent manner, perhaps by E2F1.

Some clarity has been shed on the requirement of the ASK1-p38 MAPK pathway for E2F1-induced apoptosis. The Ginsberg group [75] showed that E2F1 modulates the activity of the p38 MAPK pathway through up-regulation of p38 MAPK phosphorylation. This involves transcriptional induction of the ASK1 (apoptosis signal-regulating kinase 1) gene (also known as MAP3K5), a member of the mitogen-activated protein kinase family that phosphorylates p38 MKKs. E2F1 directly binds to the ASK1 promoter [76]. DNA microarray analysis revealed MAP3K5 up-regulation following E2F1 over-expression in p53-positive U-2OS osteosarcoma [77] and in p53-deficient melanoma cells [16]. MAPKs have been implicated in p53-independent apoptosis induced by diverse stimuli. For example, MAP3K5 has been shown to mediate rapamycin-induced apoptosis via phosphorylation of c-Jun in p53-negative mouse embryonic fibroblasts [78], whereas suppression of kinase activity and ASK1-JNK signalling through the interaction of ASK1 with p21cip1 in p53 wild-type cells [79] leads to apoptosis inhibition. The relevance of the ASK1-p38 connection for E2F1-induced apoptosis is also evident from data demonstrating that E2F1 regulates the activity of the p38 signalling inhibitor Wip1. Knock down of Wip1 has been shown to enhance E2F1-dependent apoptosis [75].

Further insight into the mechanism by which E2F1 directs p53 activity towards apoptosis comes from studies showing that E2F1 up-regulates the expression of proapoptotic cofactors of p53, JMY, TP53INP1 and the apoptosis-stimulating proteins of p53 (ASPP) family members ASPP1 and ASPP2 by a direct transcriptional mechanism [80, 81]. TP53INP1 was shown to mediate phosphorylation of p53 on serine 46. Both ASPP proteins modulate the cellular apoptotic threshold by direct interaction with p53, thereby enhancing the DNA binding and transactivation function of p53 on the promoters of proapoptotic genes in vivo [82]. Herein, ASPP specifically renders p53-guided apoptosis by stimulation of the p53-regulated promoters Bax and PIG-3. While these studies provide additional pathways by which E2F1 co-operates with p53 to induce apoptosis, the ASPP promoters can also be transactivated by E2F1 in a p53-deficient context as shown by ChIP [80] and reporter gene assays [80, 83]. Since ASPP1 and ASPP2 are specific activators of the apoptotic function of all p53 family members, E2F1 may use this pathway to increase the apoptotic function of p63 and p73 independently of p53. This ambivalence seems to be a common concept for death proteins since it has also been observed for other apoptosis factors such as SIVA and MAP3K5. SIVA seems to link the E2F/ARF/p53- and the p53-independent apoptotic pathway [72].

In a functional so-called technical knockout approach using the Saos-2 ER-E2F1 cell line, where E2F1 activity can be conditionally induced on 4-hydroxytamoxifen treatment, several other potential death targets of E2F1 were identified such as Galectin-1 (or LGALS1) [17]. E2F1-induced apoptosis was significantly abolished when Galectin-1 antisense cDNA was introduced into p53-negative cells.
Galectin-1 has been shown to play a key role in tumour-immune escape by killing antitumour effector T cells. It sensitizes, for example, human-resting T cells to Fas (CD95)/caspase-8–mediated cell death [84]. Consistently, E2F1 enhances Fas signalling in T cells [85]. E2F1 was earlier shown to be essential for T-cell apoptosis by a process called T-cell receptor (TCR) activation-induced cell death (AICD). Lissy et al. [86] demonstrated that T cells were protected from TCR-mediated apoptosis by disruption of the E2F1 gene or p73. Together these findings support a function for E2F1 in balancing life and death of immune effector cells.

Inhibition of survival signalling pathways

Beside direct activation of various apoptosis-related genes described earlier, a second major mechanism by which E2F1 sensitizes cells to apoptosis is via inhibition of antiapoptotic signalling. One example is the inhibition of NF-κB that functions in most cases as a survival signal. Activation of TNFR in response to TNF results in the stimulation of NF-κB via TRAF2, which contributes to the inhibition of cell death [87]. Importantly, E2F1 expression sensitizes cells to apoptosis by down-regulation of TRAF2 protein levels, thereby inhibiting antiapoptotic NF-κB signalling [88].

The general principle of blocking survival factors by E2F1 to induce apoptosis is supported by the data from two other studies [89, 90]. They reported that over-expression of E2F1 suppresses the expression of the apoptotic antagonists Bcl-2 and its family member Mcl-1 which leads to apoptosis. In both cases, transcriptional repression is direct and dependent on the DNA-binding domain of E2F1. The E2F1-induced decrease in Bcl-2 and Mcl-1 levels occurs independently of the ARF/p53 pathway. This scenario renders cells susceptible to apoptosis by altering the balance between anti- and proapoptotic Bcl-2 family members in favour of those that induce cell death. Together with the induction of BH3-only proteins and other proapoptotic Bcl-2 family members by E2F1, these findings support the concept that E2F1 is critical for mitochondrial apoptosis–caspase-dependent as well as caspase-independent.

There is increasing evidence that execution of the apoptotic program is achieved by an intense cross-talk between the mitochondria and the endoplasmic reticulum (ER) (reviewed in ref. [91]). A number of molecules involved in mitochondrial apoptosis are also involved in ER stress-induced cell death, suggesting that the ER might regulate apoptosis by sensitizing the mitochondria by a number of extrinsic and intrinsic stimuli, as well as by inducing apoptosis [92]. Bcl-2 family members, for example, localize to the ER membrane, thereby disrupting ER homeostasis by altering ER membrane permeability as a result of mitochondrial dysfunction [93]. Unfolded protein response–mediated cell survival or cell death is regulated by the balance between the ER chaperones GRP78 and Gadd153. E2F1 can down-regulate the expression of GRP78 (also known as BIP) [18], which normally protects cells from death induced by disturbance of ER homeostasis [94, 95]. Moreover, expression of another ER chaperon, the 94 kD glucose-regulated protein (GRP94), shown to be significantly up-regulated in human esophageal cancers [96], is also inhibited by E2F1 [18]. From these data it is evident that E2F1-mediated apoptosis involves both mitochondrial and ER-related death pathways. The whole spectrum of the proapoptotic activities of E2F1s is summarized in Figure 3.

E2F1 apoptosis and new therapeutic strategies

Since apoptosis programs can be manipulated to produce massive changes in cell death, the genes and proteins controlling apoptosis are potential drug targets. As indicated earlier, acquired apoptotic defects during cancer progression involve three types of molecular changes: (i) inactivation of proapoptotic effectors, for example, p53 or Apaf-1; (ii) activation of antiapoptotic factors, for example, Bcl-2 and (iii) reinforcement of survival signals. Thus, anticancer studies set out to either boost cell death or to impede antiapoptotic and proliferative pathways. Two observations suggest that such strategies are feasible. First, most antiapoptotic mutations act relatively upstream in the program implying that tumour cells generally retain the machinery and latent potential for
apoptosis. Second, tumour-specific alterations in apoptotic programs provide opportunities to target cell death in a selective manner. Several current strategies are discussed later.

Restoration of the apoptotic pathway, for example, by re-introduction of p53 [97] or activation of Apaf-1 [98] has been shown to increase the sensitivity of neoplastic cells to DNA-damaging agents. The ineffectiveness of a p53-based gene therapeutic strategy in certain conditions is, however, problematic (e.g. mutant p53 in tumour cells trans-dominantly impairs the function of wild-type p53) [99]. Here, genes (such as proapoptotic E2F1 targets) are particularly useful that compensate or bypass cell death defects regardless of the p53 status.

In the past few years, pre-clinical experiments mainly using E2F1 itself as anti-cancer therapeutic have been initiated. The effect of E2F1 over-expression on tumour growth has been evaluated in several types of human cancer in vitro and in vivo including glioma, melanoma, esophageal cancer, breast- and ovarian carcinoma, head and neck squamous cell cancer, gastric cancer; pancreatic carcinoma and nonsmall-cell lung cancer [54, 100–108]. These studies clearly indicate that apoptosis induction by adenoviral expressed E2F1 results in growth suppression and increased responsiveness of tumour cells to chemotherapy with relative sparing of normal tissues. This selectivity might be due to the fact that normal cells contain wild-type pRB, which likely can bind to and antagonize the activity of exogenous E2F1. Furthermore, transcription from E2F1-responsive promoters is higher in cancer cells than in normal cells. However, targeting E2F1 itself is a cumbersome mission possibly feasible for some but not suitable for all therapeutic purposes because of its dual role in cell cycle progression and apoptosis. Regarding this issue, it has been shown that a transcriptionally inactive form of E2F1 is able to induce apoptosis without activating proliferation in human coronary vascular smooth muscle cells (VSMC) [43]. Therefore, this strategy has widespread potential for preventing hyperproliferation in artherosclerosis, hypertension and restenosis after injury.

In addition to E2F1, its downstream target p73 was proven to be a valuable candidate for cancer therapy in tumour cells. Infection with an adenoviral vector encoding the beta isoform of p73 resulted in potent cytotoxicity based on a combination of cell cycle arrest and apoptosis induction [64, 109, 110]. Similar to E2F1, p73 can efficiently induce apoptosis and enhanced chemosensitivity of tumour cells primarily resistant to wild-type p53. Although this strategy warrants further consideration, the antitumoural effect exhibited by over-expression of p73, in contrast to its homolog p53, is not restricted to tumour cells.

Although present data provide compelling evidence that effectors of E2F1-induced apoptosis can significantly kill cancer cells, their role as a target for cancer treatment is only beginning to emerge. So far, in clinical studies antiapoptotic E2F1 regulated genes were used to hamper tumour growth instead of using approaches for introducing proapoptotic molecules. Antiapoptotic Bcl-2, for example, has
been targeted extensively by antisense oligonucleotide approaches which interfere with Bcl-2 activity, resulting in less Bcl-2 expression and elevated levels of apoptosis [111]. In the phase I–II study, Bcl-2 antisense oligonucleotide (G3139) treatment of chemoresistant melanoma patients led to minor to complete therapeutic response in 6 of 14 cases when the antisense therapy was combined with dacarbazine administration. In a Phase II study, Bcl-2 antisense oligonucleotide treatment of patients with multiple myeloma led to therapeutic responses in 55% of cases when combined with chemotherapy [112]. Bcl-2 antisense strategies are currently under way in Phase III clinical trials. These data underline that inhibition of antiapoptotic Bcl-2 is surely a promising treatment for otherwise resistant tumour entities.

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References

1. Ohtani K, DeGregori J, Nevins JR. Regulation of the cyclin E gene by transcription factor E2F. Proc Natl Acad Sci USA. 1995; 92: 12146–50.
2. Johnson DG, Schwarz JK, Cress WD, Nevins JR. Expression of transcription factor E2F1 induces quiescent cells to enter S phase. Nature. 1993; 365: 349–52.
3. Dyson N. The regulation of E2F by pRB-family proteins. Genes Dev. 1998; 12: 2245–62.
4. Müller H, Helin K. The E2F transcription factors: key regulators of cell proliferation. Biochim Biophys Acta. 2000; 1470: M1–2.
5. Weinberg RA. The retinoblastoma protein and cell cycle control. Cell. 1995; 81: 323–30.
6. DeGregori J, Johnson DG. Distinct and overlapping roles for E2F family members in transcription, proliferation and apoptosis. Curr Mol Med. 2006; 6: 739–48.
7. Trimarchi JM, Lees JA. Sibling rivalry in the E2F family. Nat Rev Mol Cell Biol. 2002; 3: 11–20.
8. DeGregori J. The genetics of the E2F family of transcription factors: shared functions and unique roles. Biochim Biophys Acta. 2002; 1602: 131–50.
9. Logan N, Delavaine L, Graham A, Reilly C, Wilson J, Brummelkamp TR, Hijmans EM, Bernards R, La Thangue NB. E2F-7: a distinctive E2F family member with an unusual organization of DNA-binding domains. Oncogene. 2004; 23: 5138–50.
10. Ren B, Cam H, Takahashi Y, Volkert T, Terragni J, Young RA, Dynlacht BD. E2F integrates cell cycle progression with DNA repair, replication, and G2/M checkpoints. Genes Dev. 2002; 16: 245–56.
11. Dimova DK, Stevaux O, Frolov MV, Dyson NJ. Cell cycle-dependent and cell cycle-independent control of transcription by the Drosophila E2F/RF pathway. Genes Dev. 2003; 17: 2306–20.
12. Cam H, Balcinuaine E, Blais A, Spector A, Scarpulla RC, Young R, Kluger Y, Dynlacht BD. A common set of gene regulatory networks links metabolism and growth inhibition. Mol Cell. 2004; 16: 399–411.
13. Bieda M, Xu X, Singer MA, Green R, Farnham PJ. Unbiased location analysis of E2F1-binding sites suggests a widespread role of E2F1 in human genome. Genome Res. 2006; 16: 595–605.
14. Stanelle J, Stiewe T, Theseling CC, Peter M, Pützer BM. Identification of novel E2F1-regulated genes. Nucleic Acids Res. 2002; 30: 1859–67.
15. Ma Y, Croxton R, Moorer RL, Cress WD. Identification of novel E2F1-regulated genes by microarray. Arch Biochem Biophys. 2002; 399: 212–24.
16. Jamshidi-Parsian A, Dong Y, Zheng X, Zhou HS, Zacharias W, McMasters KM. Gene expression profiling of E2F-1-induced apoptosis. Gene. 2005; 344: 67–77.
17. Li Z, Stanelle J, Leurs C, Hanenberg H, Pützer BM. Selection of novel mediators of E2F1-induced apoptosis through retroviral expression of an antisense cDNA library. Nucleic Acids Res. 2005; 33: 2813–21.
18. Li Z, Kreutz M, Mikkat S, Mise N, Glocker MO, Pützer BM. Proteomic analysis of the E2F1 response in p53-negative cancer cells: new aspects in the regulation of cell survival and death. Proteomics. 2006; 6: 5735–45.
19. Phillips AC, Vousden KH. E2F1 induced apoptosis. Apoptosis. 2001; 6: 173–82.
20. Ginsberg D. E2F1 pathways to apoptosis. FEBS Lett. 2002; 529: 122–5.
21. Sears RC, Nevins JR. Signaling networks that link cell proliferation and cell fate. J Biol Chem. 2002; 277: 11617–20.
Induction of DNA synthesis and apoptosis are separable functions of E2F-1. *Genes Dev.* 1997; 11: 1853–63.

Hsieh J-K, Fredersdorf S, Kouzarides T, Martin K, Lu X. E2F-1 induced apoptosis requires DNA binding but not transactivation and is inhibited by the retinoblastoma protein through direct interaction. *Genes Dev.* 1997; 11: 1840–52.

Holmberg C, Helin K, Sehested M, Karlstrom O. E2F-1 induced p53-independent apoptosis in transgenic mice. *Oncogene.* 1998; 17: 143–55.

Haas-Kogan DA, Kogan S, Levi D, Dazin P, T’Ang A, Fung Y K, Israel MA. Inhibition of apoptosis by the retinoblastoma gene product. *EMBO J.* 1995; 14: 461–72.

Stiewe T, Pützer BM. Role of the p53 homolog p73 for E2F1-induced apoptosis. *Nat Genet.* 2000; 26: 464–9.

Irwin M, Martin MC, Phillips AC, Seelan RS, Smith DI, Liu W, Flores ER, Tsai KY, Jacks T, Vousden KH, Kaelin WG Jr. Role for the p53 homologue p73 in E2F-1-induced apoptosis. *Nature.* 2000; 407: 645–8.

Moroni MC, Hickman ES, Denchi EL, Caprara G, Colli E, Cecconi F, Müller H, Helin K. Apaf-1 is a transcriptional target for E2F1 and p53. *Nat Cell Biol.* 2001; 3: 552–8.

Stanelle J, Stiewe T, Rödicker F, Köhler K, Theseling CC, Pützer BM. Mechanisms of E2F1-induced apoptosis in primary vascular smooth muscle cells. *Cardiovasc Res.* 2003; 59: 512–9.

Bell LA, O’Prey J, Ryan KM. DNA-binding independent cell death from a minimal proapoptotic region of E2F-1. *Oncogene.* 2006; 25: 5656–63.

Hallstrom TC, Nevins JR. Specificity in the activation and control of transcription factor E2F-dependent apoptosis. *Proc Natl Acad Sci USA.* 2003; 100: 10648–53.

Chausseped M, Ginsberg D. Transcriptional regulation of AKT activation by E2F. *Mol Cell.* 2004; 16: 831–7.

Moon NS, Di Stefano L, Dyson N. A gradient of epidermal growth factor receptor signaling determines the sensitivity of rbf1 mutant cells to E2F-dependent apoptosis. *Mol Cell Biol.* 2006; 26: 7601–15.

Lin WC, Lin FT, Nevins JR. Selective induction of E2F1 in response to DNA damage, mediated by ATM-dependent phosphorylation. *Genes Dev.* 2001; 15: 1833–44.

Pedicini N, Ianari A, Costanzo A, Belloni L, Gallo R, Cimino L, Porcellini A, Screpanti I, Balsano C, Alesse E, Gulino A, Leverero M. Differential regulation of E2F1 apoptotic target genes in response to DNA damage. *Nat Cell Biol.* 2003; 5: 552–8.

Ianari A, Gallo R, Palma M, Alesse E, Gulino A. Specific role for p300/CREB-binding protein-associated factor activity in E2F1 stabilization in response to DNA damage. *J Biol Chem.* 2004; 279: 30830–5.
51. Banerjee D, Schnieders B, Fu J, Ashikari D, Zhao S-C, Bertino J. Role of E2F-1 in chemosensitivity. Cancer Res. 1998; 58: 4292–6.

52. Meng R, Phillips P, El-Deiry W. p53-independent increase in E2F-1 expression enhances the cytotoxic effects of etoposide and adriamycin. Int J Oncol. 1999; 14: 5–14.

53. Pruschy M, Wirbelauer C, Glanzmann C, Bodis S, Krek W. E2F-1 has properties of a radiosensitizer and its regulation by cyclin A is required for cell survival of fibrosarcoma cells lacking p53. Cell Growth Differ. 1999; 10: 141–6.

54. Rödicker F, Stiewe T, Zimmermann S, Pützer BM. Therapeutic efficacy of E2F-1 in pancreatic cancer correlates with TP73 induction. Cancer Res. 2001; 61: 7052–5.

55. Polager S, Kalma Y, Berkovich E, Ginsberg D. E2Fs up-regulate expression of genes involved in DNA replication, DNA repair and mitosis. Oncogene. 2002; 21: 437–46.

56. Hao H, Dong YB, Bowling MT, Zhou HS, Real PJ, Sanz C, Gutierrez O, Pipoan C, Zubiaga AM, Fernandez-Luna JL. Transcriptional activation of the proapoptotic bik gene by E2F-2 proteins in cancer cells. FEBS Lett. 2006; 580: 5905–9.

57. Bouillet P, Strasser A. Bax and Bak: back-bone of T cell death. Nat Immunol. 2002; 3: 893–4.

58. Chen L, Willis SN, Wei A, Smith BJ, Fletcher JJ, Hinds MG, Colman PM, Day CL, Adams JM, Huang DC. Differential targeting of prosurvival Bcl-2 proteins by their BH3-only ligands allows complementary apoptotic function. Mol Cell. 2005; 17: 393–403.

59. Stanelle J, Tu-Rapp H, Pützer BM. A novel mitochondrial protein DIP mediates E2F1-induced apoptosis independently of p53. Cell Death Differ. 2005; 12: 347–57.

60. Xie W, Jiang P, Miao L, Zhao Y, Zhimin Z, Qing L, Zhu WG, Wu M. Novel link between E2F1 and Smac/DIABOLO: proapoptotic Smac/DIABOLO is transcriptionally upregulated by E2F1. Nucleic Acids Res. 2006; 34: 2046–55.

61. Nahle Z, Polakoff J, Davuluri RV, Mc Currach ME, Jacobson MD, Narita M, Zhang MQ, Lazebnik Y, Bar-Sagi D, Lowe SW. Direct coupling of the cell cycle and cell death machinery by E2F. Nat Cell Biol. 2002; 4: 859–64.

62. Fortin A, MacLaurin JG, Arbour N, Cregan SP, Kushwaha N, Callaghan SM, Park DS, Albert PR, Slack RS. The proapoptotic gene SIVA is a direct transcriptional target for the tumor suppressors p53 and E2F1. J Biol Chem. 2004; 279: 28706–14.

63. Xue L, Chu F, Cheng Y, Sun X, Borthakur A, Ramara M, Pandey P, Wu M, Schlossman SF, Prasad KVS. SIVA binds to and inhibits BCL-XL-mediated protection against UV radiation-induced apoptosis. Proc Natl Acad Sci USA. 2002; 99: 6925–30.

64. Qin LF, Lee TK, Ng IO. Gene expression profiling by cDNA array in human hepatoma cell line in response to cisplatin treatment. Life Sci. 2002; 70: 1677–90.

65. Hershko T, Ginsberg D. Up-regulation of Bcl-2 homology 3 (BH3)-only proteins by E2F1 mediates apoptosis. J Biol Chem. 2004; 279: 8827–34.

66. Tuve S, Racek T, Niemetz A, Schultz J, Soengas MS, Pützer BM. Adenovirus-mediated TA-p73 gene transfer increases chemosensitivity of human malignant melanomas. Apoptosis. 2006; 11: 235–45.
is a direct E2F1 target gene. Biochem J. 2006; 396: 547–56.

77. Müller H, Bracken AP, Vernell R, Moroni MC, Christians F, Grassilli E, Prosperi E, Vigo E, Oliner JD, Helin K. E2Fs regulate the expression of genes involved in differentiation, development, proliferation, and apoptosis. Genes Dev. 2001; 15: 267–85.

78. Huang S, Shu L, Dilling MB, Easton J, Harwood FC, Ichijo H, Houghton PJ. Sustained activation of the JNK cascade and rapamycin-induced apoptosis are suppressed by p53/p21Cip1. Mol Cell. 2003; 11: 1491–501.

79. Asada M, Yamada T, Ichijo H, Delia D, Miyazono K, Fukumuro K, Mizutani S. Apoptosis inhibitory activity of cytoplasmic p21(Cip1/WAF1) in monocytic differentiation. EMBO J. 1999; 18: 1223–34.

80. Fogal V, Kartasheva NN, Trigante G, Lianos S, Yap D, Vousden KH, Lu X. ASPP1 and ASPP2 are new transcriptional targets of E2F. Cell Death Differ. 2005; 12: 369–76.

81. Hershko T, Chaussee M, Poll M, Oren G, Ginsberg D. The E2F-1 transcription factor promotes caspase-8 and bid expression, budding, and fission. J Biol Chem. 2002; 277: 21836–42.

82. Matarrese P, Tinari A, Mormone E, Bianco GA, Toscano MA, Ascione B, Rabinovich GA, Malorni W. Galectin-1 sensitizes resting human T lymphocytes to Fas (CD95)-mediated cell death via mitochondrial hyperpolarization, budding, and fission. J Biol Chem. 2005; 280: 6969–85.

83. Wang XF, Liu GZ, Song AL, Chen RF, Li HY, Liu Y. Expression and significance of heat shock protein 70 and glucose-regulated protein 94 in human esophageal carcinoma. World J Gastroenterol. 2005; 11: 429–32.

84. Roemer K, Friedmann T. Mechanisms of action of the p53 tumor suppressor and prospects for cancer gene therapy by reconstitution of p53 function. J Cell Biochem. 2000; 77: 396–408.

85. Wang XP, Liu GZ, Song AL, Chen RF, Li HY, Liu Y. Expression and significance of heat shock protein 70 and glucose-regulated protein 94 in human esophageal carcinoma. World J Gastroenterol. 2005; 11: 429–32.

86. Roemer K, Friedmann T. Mechanisms of action of the p53 tumor suppressor and prospects for cancer gene therapy by reconstitution of p53 function. Ann N Y Acad Sci. 1994; 716: 265–80.

87. Soengas MS, Capodieci P, Polsky D, Mora J, Esteller M, Opitz-Araya X, McCombie R, Herman JG, Gerald WL, Lazebnik YA, Cordon-Cardo C, Lowe SW. Inactivation of the apoptosis effector Apaf-1 in malignant melanoma. Nature. 2001; 409: 207–11.

88. Poljak K, Waldman T, He TC, Kinzler KW, Vogelstein B. Genetic determinants of p53-induced apoptosis and growth arrest. Genes Dev. 1996; 10: 1945–52.

89. Eischen CM, Packham G, Nip J, Fee BE, Hiebert SW, Zambetti GP, Cleveland JL. Bcl-2 is an apoptotic target suppressed by both c-Myc and E2F-1. Oncogene. 2001; 20: 6983–93.

90. Croxton R, Ma Y, Song L, Haura EB, Cress WD. Direct repression of the Mcl-1 promoter by E2F1. Oncogene. 2002; 21: 1359–69.

91. Kim R, Emi M, Tanabe K, Murakami S. Role of the unfolded protein response in cell death. Apoptosis. 2006; 11: 5–13.

92. Rao RV, Castro-Obregon S, Frankowski H, Schuler M, Stoka V, del Rio G, Bredesen DE, Ellerby HM. Coupling endoplasmic reticulum stress to the cell death program. An Apaf-1-independent intrinsic pathway. J Biol Chem. 2002; 277: 21836–42.

93. Scorrano L, Oakes SA, Opferman JT, Cheng EH, Soricelli MD, Pozzan T, Korsmeyer SJ. BAX and BAK regulation of endoplasmic reticulum Ca2+: a control point for apoptosis. Science. 2003; 300: 135–9.

94. Reddy RK, Mao C, Baumeister P, Austin RC, Kaufman RJ, Lee AS. Endoplasmic reticulum chaperone protein GRP78 protects cells from apoptosis induced by topoisomerase inhibitors: role of ATP binding site in suppression of caspase-7 activation. J Biol Chem. 2003; 278: 20915–24.

95. Miyake H, Hara I, Arakawa S, Kamidono S. Stress protein GRP78 prevents apoptosis induced by calcium ionophore, ionomycin, but not by glycosylation inhibitor, tunicamycin, in human prostate cancer cells. J Cell Biochem. 2000; 77: 396–408.

96. Wang XP, Liu GZ, Song AL, Chen RF, Li HY, Liu Y. Expression and significance of heat shock protein 70 and glucose-regulated protein 94 in human esophageal carcinoma. World J Gastroenterol. 2005; 11: 429–32.

97. Roemer K, Friedmann T. Mechanisms of action of the p53 tumor suppressor and prospects for cancer gene therapy by reconstitution of p53 function. Ann N Y Acad Sci. 1994; 716: 265–80.

98. Soengas MS, Capodieci P, Polsky D, Mora J, Esteller M, Opitz-Araya X, McCombie R, Herman JG, Gerald WL, Lazebnik YA, Cordon-Cardo C, Lowe SW. Inactivation of the apoptosis effector Apaf-1 in malignant melanoma. Nature. 2001; 409: 207–11.

99. Poljak K, Waldman T, He TC, Kinzler KW, Vogelstein B. Genetic determinants of p53-induced apoptosis and growth arrest. Genes Dev. 1996; 10: 1945–52.

100. Fueyo J, Gonzalez-Manzano C, Yung WKA, Liu TJ, Alemany R, McDonnell TJ, Shi X, Rao JS, Levin VA, Kyritsis AP. Overexpression of E2F-1 in glioma triggers apoptosis and suppresses tumor growth in vitro and in vivo. Nat Med. 1998; 4: 685–90.
101. Dong YB, Yang HL, Elliott MJ, Liu TJ, Stilwell A, Atienza C, McMasters KM. Adenovirus-mediated E2F-1 gene transfer efficiently induces apoptosis in melanoma cells. Cancer. 1999; 86: 2021-33.

102. Yang H, Dong Y, Elliott M, Liu T, Atienza CJ, Stilwell A, McMasters KM. Adenovirus-mediated E2F-1 gene transfer inhibits MDM2 expression and efficiently induces apoptosis in MDM2-overexpressing tumor cells. Clin Cancer Res. 1999; 5: 2242–50.

103. Yang H, Dong Y, Elliott M, Liu T, McMasters K. Caspase activation and changes in Bcl-2 family member protein expression associated with E2F-1 mediated apoptosis in human esophageal cancer cells. Clin Cancer Res. 2000; 6: 1579–89.

104. Hunt KK, Deng J, Liu TJ, Wilson-Heiner N, Swisher SG, Clayman G, Hung MC. Adenovirus-mediated overexpression of the transcription factor E2F1 induces apoptosis in human breast cancer and ovarian carcinoma cell lines and does not require p53. Cancer Res. 1997; 57: 4722–6.

105. Liu T, Wang M, Breau R, Henderson Y, El-Naggar A, Steck K, Sicard MW, Clayman GL. Apoptosis induction by E2F-1 via adenoviral-mediated gene transfer results in growth suppression of head and neck squamous cell carcinoma cell lines. Cancer Gene Ther. 1999; 6: 163–71.

106. Atienza C, Elliott MJ, Dong YB, Yang HI, Stilwell A, Liu TJ, McMasters KM. Adenovirus-mediated E2F-1 gene transfer induces an apoptotic response in human gastric carcinoma cells that is enhanced by cyclin dependent kinase inhibitors. Int J Mol Med. 2000; 6: 55–63.

107. Kuhn H, Liebers U, Gessner C, Schumacher A, Witt C, Schauer J, Kovesdi I, Wolff G. Adenovirus-mediated E2F-1 gene transfer in nonsmall-cell lung cancer induces cell growth arrest and apoptosis. Eur Respir J. 2002; 20: 703–9.

108. Parr MJ, Manome Y, Tanaka T, Wen P, Kufe DW, Kaelin WG Jr, Fine HA. Tumor-selective transgene expression in vivo mediated by an E2F-responsive adenoviral vector. Nat Med. 1997; 3: 1145–9.

109. Rödicker F, Pützer BM. p73 is effective in p53-null pancreatic cancer cells resistant to wild-type TP53 gene replacement. Cancer Res. 2003; 63: 2737–41.

110. Das S, Nama S, Antony S, Somasundaram K. p73beta-expressing recombinant adenovirus: a potential anticancer agent. Cancer Gene Ther. 2005; 12: 417–26.

111. Schlagbauer-Wadl H, Klosner G, Heere-Ress E, Waltering S, Moll I, Wolff K, Pehamberger H, Jansen B. Bcl-2 antisense oligonucleotides (G3139) inhibit Merkel cell carcinoma growth in SCID mice. J Invest Dermatol. 2000; 114: 725–30.

112. Badros AZ, Goloubeva O, Rapaport AP, Ratterree B, Gahres N, Meisenberg B, Takebe N, Heyman M, Zwiebel J, Steicher H, Gocke CD, Tomic D, Flaws JA, Zhang B, Fenton RG. Phase II study of G3139, a Bcl-2 antisense oligonucleotide, in combination with dexamethasone and thalidomide in relapsed multiple myeloma patients. J Clin Oncol. 2005; 23: 4089–99.