FEBS Letters 529 (2002) 122–125 FEBS 26496

Minireview

E2F1 pathways to apoptosis

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Received 12 August 2002; accepted 16 August 2002

First published online 27 August 2002

Edited by Gunnar von Heijne

Abstract The E2F family of transcription factors plays a pivotal role in the regulation of cell proliferation and their activity is often deregulated in human tumors. Recent studies demonstrate that E2F1 can induce both proliferation and apoptosis. E2F1-induced apoptosis occurs via multiple pathways, some of which induce stabilization and activation of the tumor suppressor p53. The pro-apoptotic activity of E2F1 suggests that its deregulation constitutes an oncogenic stress that may target pre-malignant cells to undergo apoptosis, thus preventing tumor development.

Key words: E2F; Apoptosis; p14ARF; p53; p73; ApafI

1. Introduction

The E2F family of transcription factors comprises six structurally related E2Fs (E2F1–6) that function as heterodimers with members of the DP family (DP-1 and DP-2). Initial studies implicated E2F/DP heterodimers mainly in transcriptional activation of genes required for cell cycle progression. However, it is currently clear that transcriptional activation of cell cycle-related genes is only one facet of E2F activity: data accumulated over the last few years demonstrate that E2Fs function in both transactivation and repression of gene expression. Furthermore, it is now clear that E2Fs have important roles in regulating both cell proliferation and antiproliferative processes such as apoptosis and senescence.

E2F transcriptional activity is modulated by multiple mechanisms, including negative regulation by interaction with the product of the Rb tumor suppressor gene, pRB, and its related proteins p107 and p130, collectively referred to as pocket proteins (reviewed in [1]). This association not only inhibits E2F-induced transactivation but also actively represses transcription upon interaction of the E2F-associated pocket proteins with histone deacetylases (HDACs), hSWI/SNF nucleosome remodeling complex, and polycomb group proteins [2]. Formation of pocket proteins/E2F containing complexes is cell cycle-regulated. Specifically, cyclins expressed at the G1-phase of the cell cycle positively regulate their associated kinases, the cyclin-dependent kinases (Cdk), which phosphorylate the pocket proteins [3,4], resulting in sequential dissociation of HDAC/RB/E2F complexes. This leads to derepression as well as activation of E2F-regulated genes and an ensuing S-phase entry. Deregulated E2F activity occurs in the majority of human tumors, via a number of different mechanisms. These include: functional loss of pRB, the negative regulator of E2F activity; amplification of cyclin D, which promotes phosphorylation of pRB; loss of p16, a Cdk inhibitor which inhibits the phosphorylation of pRB; or expression of the HPV oncoprotein E7, which dissociates RB/E2F complexes [4].

Based on structure, transcriptional properties and association with pocket proteins, the E2F family can be divided into three distinct subgroups: E2F1, E2F2 and E2F3, E2F4 and E2F5, and E2F6. E2F1, E2F2 and E2F3 associate exclusively with pRB and are potent transcriptional activators. In contrast, E2F4, which associates with RB, p107 and p130, and E2F5, which associates with p130, seem to be primarily involved in the active repression of E2F-responsive genes [5]. E2F6 does not interact with pocket proteins and functions as negative regulator of E2F-dependent transcription via complexing with chromatin modifiers [6,7].

Exogenous expression of E2F1, E2F2 or E2F3 in quiescent cells results in S-phase entry [8,9]. In addition, E2F1 has an apparent unique ability to induce apoptosis. This review focuses on E2F1-induced apoptosis. For recent reviews discussing other biological functions of E2F family members see [5,10].

2. E2F1 induces apoptosis

A variety of experiments implicate E2F1 in induction of apoptosis (reviewed in [11]): ectopic expression of E2F1 leads to apoptosis in tissue culture cells [12–14] and transgenic mice [15–17]. A physiological role for E2F1-mediated apoptosis is suggested by the observation that mice deficient in E2F1 have an excess of mature T cells due to a defect in thymocyte apoptosis [18]. In addition, mice deficient in both Rb and E2F1 demonstrate significant suppression of both the aberrant apoptosis and the S-phase entry observed in mice carrying only homozygous Rb mutation [19]. Similarly, inactivation of Rb in mouse brain epithelium induces aberrant proliferation and apoptosis that are inhibited by E2F1 deficiency [20], implicating E2F1 in Rb-dependent apoptosis. Interestingly, also E2F3 deficiency, but not E2F2 deficiency, suppresses the inappropriate apoptosis arising in the Rb mutant mice embryos, providing evidence for a role of E2F3 in the apoptosis that occurs in the absence of Rb [21,22]. Ectopic expression of E2F2 and E2F3 was reported to induce apoptosis in some experimental systems [9] but not in others [8,23]. It remains to be deter-

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0014-5793/02/$22.00
PII: S0014-5793(02)03270-2

mined whether the observed E2F2 and E2F3-induced apoptosis is E2F1-dependent.

The induction of apoptosis by E2F1, as for c-Myc, is abrogated by growth factors [12]. In fact, the ability of Myc to induce apoptosis is E2F1-dependent and is markedly reduced in cells deleted for E2F1 [24].

Experiments using tissue culture cells as well as transgenic mice demonstrate that E2F1-induced apoptosis is mediated by both p53-dependent [12,13,25] and p53-independent [16,26] pathways.

3. The role of p53 in E2F1-induced apoptosis

Ectopic expression of E2F1 induces p53 accumulation [14,27] and one mechanism underlying this phenomenon is the direct transactivation of the p14ARF tumor suppressor gene (p19ARF in rodents) by E2F1 [28,29]. ARF interacts with the Mdm2 E3 ubiquitin ligase, and inhibits the ability of mdm2 to target p53 to ubiquitination and subsequent degradation [30]. Thus, the E2F1-induced increase in ARF levels leads to p53 stabilization and activation.

In addition, E2F1 increases p53 levels and p53-dependent apoptosis also in the absence of ARF, suggesting the existence of additional, ARF-independent, functional links between E2F1 and p53. A number of recent studies demonstrate that RB inactivation or E2F1 over-expression leads to apoptosis that is inhibited by loss of p53 but not by loss of ARF [31–33]. In fact, in some cases loss of ARF even enhances E2F1-induced apoptosis [31,33]. This enhancement is in agreement with the ability of ectopically expressed ARF to inhibit E2F1-induced apoptosis [34] and target E2F1 to degradation [35].

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 [31,36]. Induction of both apoptosis and p53 phosphorylation by E2F1 are abolished by caffeine, an inhibitor of the ATM and ATR protein kinases ([36] and D. Johnson, personal communication). These data implicate kinases of the ATM family in E2F1-mediated apoptosis. However, the mechanism governing their activation by E2F1 remains to be determined.

E2F1 interacts with p53 and a recent study demonstrates that this interaction occurs via the cyclin A binding domain of E2F1 and enhances the apoptotic activity of p53 [37]. This function of E2F1 does not require DNA binding or transcriptional activity and it is shared by E2F2 and E2F3 [37].

Taken together, the existing data strongly suggest that E2F1 affects p53-dependent apoptosis through a number of pathways. One of these pathways involves activation of ARF, another relies on physical interaction with p53, while yet another is caffeine-sensitive. The exact nature of the two latter mechanisms awaits further studies.

4. Additional pathways mediating E2F1-induced apoptosis

The p53 homolog p73 also plays a role in E2F1-induced apoptosis. E2F1 directly activates transcription of p73, leading to activation of p53-responsive target genes and apoptosis [38,39]. Disruption of p73 function, by dominant negative p73 mutants or gene targeting, inhibits E2F1-induced apoptosis [38,39].

Stimulation of the T-cell receptor (TCR) on mature T cells causes their apoptosis by a process called TCR-activation-induced cell death (TCR-AICD). E2F1 and p73 are required for TCR-AICD, which is a physiological and naturally occurring apoptotic process [40]. However, although E2F1 is clearly involved in AICD, it has not been demonstrated whether it is required for induction of p73 in this process.

E2F1 also directly activates the expression of the Apaf-1 gene encoding for apoptosis protease-activating factor 1 [41,42]. When induced, ApafI assembles with cytochrome c, a mitochondrial protein released upon apoptotic signals, and activates procaspase 9, leading to the activation of downstream effector caspases, including caspase-3, caspase-6 and caspase-7. Induction of E2F1 activity results in an increase of mRNA and protein levels of ApafI and a concomitant activation of caspases-9, -3, -6 and possibly also caspase-7 [41,42]. E2F1-induced apoptosis is significantly reduced by inhibitors of caspase activity or by gene disruption of ApafI, indicating that the E2F1/ApafI/caspases pathway is an important mediator of E2F1-induced apoptosis [41,42]. Additional support for this notion comes from the study of RB null mice. $RB^{-/-}$ embryos exhibit increased levels of ApafI [41] and analysis of compound mutants lacking both Rb and ApafI demonstrates that ApafI is required for apoptosis induced by RB deficiency in some but not all tissues [43].

ApafI is a direct transcriptional target of p53 [41], raising the possibility that its transactivation by E2F1 is indirect. However, this is most probably not the case, since E2F1 activates deleted versions of the ApafI promoter that are not activated by p53 [41]. Furthermore, E2F1 binds the ApafI promoter and transactivates the ApafI gene in cells lacking p53 [42].

Increased E2F1 activity also results in release of cytochrome c from mitochondria to the cytoplasm. However, such release has been observed only in cell lines with wt p53 but not in a cell line lacking functional p53 [41,42], and the mechanism underlying E2F1-induced cytochrome c release has not been elucidated yet.

DNA microarray studies demonstrate that ectopic expression of E2Fs up-regulates expression of pro-apoptotic members of the Bcl-2 family, including Bok, Bad, Bak and Bid1 [44–46]. The validity of these DNA microarray data and the importance of these pro-apoptotic genes in E2F1-induced apoptosis are currently being studied. In addition, DNA microarray analysis indicates that ectopic expression of E2Fs up-regulates expression of caspase-3 and caspase-7 [44,47], and a recent study demonstrates that E2F1 directly regulates the expression of several members of the caspase family (S. Lowe, personal communication).

Interestingly, studies using E2F1 mutants demonstrate that although its DNA-binding activity is required, transcriptional transactivation is not necessary for the induction of apoptosis by E2F1 [26,48]. These observations indicate that alleviation of E2F-dependent transcriptional repression contributes to E2F1-induced apoptosis. Additional studies are required to determine whether E2F1-mediated repression or activation regulate expression of specific apoptosis-related genes.

5. E2F1 inhibits anti-apoptotic signals

Another mechanism by which deregulated E2F1 triggers apoptosis and sensitizes cells to pro-apoptotic stimuli involves disruption of NF-κB signaling [49,50]. The NF-κB transcription factor is a major regulator of cell survival and its induc-

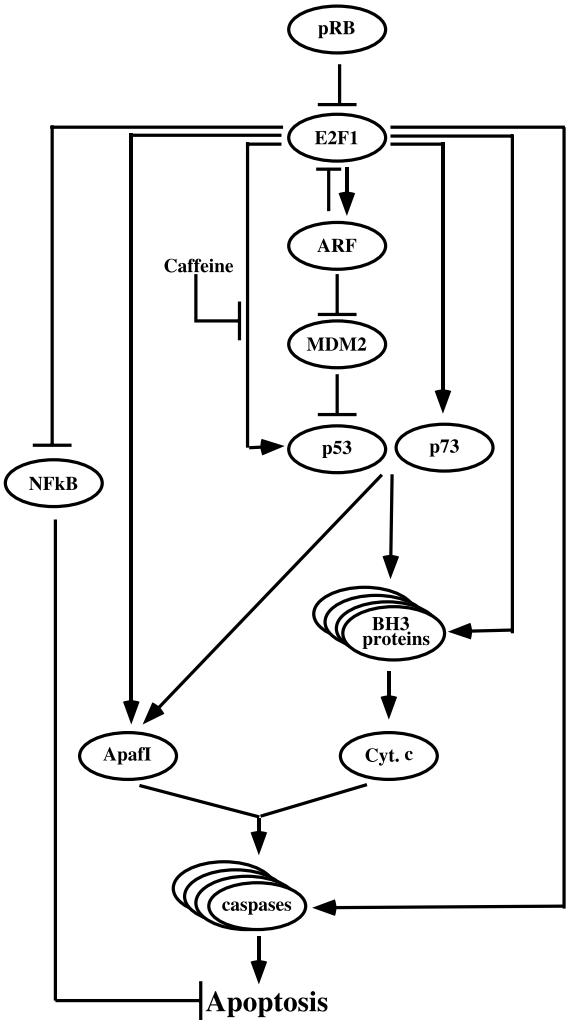

Fig. 1. Pathways of E2F1-induced apoptosis.

tion in response to death-promoting stimuli confers resistance to apoptosis. The anti-apoptotic activity of NF-κB is mediated by transcriptional activation of various anti-apoptotic genes (reviewed in [51]).

E2F1 was shown to down-regulate TRAF2 protein levels, thus leading to impaired TNF-receptor-mediated NF-κB and JNK activation in response to TNFα [49]. The molecular basis for this down-regulation of TRAF2 protein levels remains unclear but it does not require E2F1-dependent transactivation, since this effect is exerted also by a transactivation-deficient mutant of E2F1 [49].

Rel family proteins dimerize to form the functional NF-κB transcription factor [51] and a recent study showed that E2F1 competes with p50 for RelA/p65 binding, thus inhibiting NF-κB DNA binding activity [50]. These data provide another mechanism for E2F1-mediated inhibition of NF-κB activity.

6. E2F1 sensitizes cells to apoptotic stimuli

Overexpression of E2F1 in a variety of cell types sensitizes cells to apoptosis when they are treated with ionizing radiation or chemotherapeutic drugs such as the topoisomerase II inhibitors, etoposide and adriamycin [52,53]. A direct role for E2F1 in the response to DNA damage is suggested by its up-regulation after DNA damage [54–56]. This stress-induced increase in E2F1 protein levels is due to protein stabilization that is mediated by an ATM-induced phosphorylation [57]. However, it is not clear whether the stress-induced E2F1 is transcriptionally active. It does not transactivate a number of known E2F targets [56] and it remains to be determined whether it can regulate expression of apoptosis-related genes. Thus, the molecular mechanism(s) underlying the ability of E2F1 to sensitize cells to apoptotic stimuli are currently not fully understood. Nevertheless, it is well established that the pRB pathway is functionally inactivated in most human cancers, leading to deregulated E2F activity. Therefore, the ability of E2F1 to sensitize cells to death in response to genotoxic stress might play an important role in the increased sensitivity of tumor cells to chemo- and radiotherapy.

Overall, the induction of apoptosis by deregulated E2F1 is by now well established and the studies discussed above provide a number of pathways mediating this activity (Fig. 1). Nevertheless, a number of key questions are still unanswered. Most probably we do not yet have the full spectrum of apoptosis-related genes that are regulated by E2F1. The large-scale screens for E2F-regulated genes provide new and interesting possible links between E2F1 and apoptotic pathways that will undoubtedly be followed by detailed studies and will provide novel insights into the apoptotic potential of E2F1. Additional issues that require further elucidation include the role of E2F3 and the importance of E2F-mediated transcriptional repression in apoptosis. Importantly, keeping in mind the pivotal role of the E2F family in regulating cell proliferation, it is imperative to better understand the mechanism(s) determining whether the final outcome of E2F activity will be proliferation or death.

Acknowledgements: D.G. is an incumbent of the Recanati Career Development chair of cancer research. I thank M. Chaussepied for reading this manuscript, D. Johnson and S. Lowe for sharing unpublished data and 'Yad Abraham Research Center for Diagnostics and Therapy' for support. I apologize to many colleagues for not citing all the papers discussing E2F-induced apoptosis, owing to space limitations.

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