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Translating DNA damage into cancer cell death—A roadmap for E2F1 apoptotic signalling and opportunities for new drug combinations to overcome chemoresistance

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ABSTRACT

The cellular transcription factor E2F1 has been identified as a tumor suppressor regulating the activities of p53 and its homologue TAp73, and promoting apoptosis by the activation of a plethora of death pathways. More than 15 years of experimentation recognized E2F1 as the key player in apoptosis induced by DNA damage in all types of human cancer. This occurs by several mechanisms that affect RB-E2F1 interaction, E2F1 stability and its binding to promoters of E2F1-regulated genes. Recent progress has been made in revealing new proapoptotic genes regulated by E2F1 and it seems that many still remain to be discovered. However, whereas in the past one focused mainly on identifying E2F1 target genes translating cellular stress signals into cell death, today the DNA damage-induced regulatory network governing E2F1's ability to induce apoptosis is rapidly gaining attention as well. Notably, the lately uncovered role of pRB and E2F3 in triggering E2F1-dependent apoptosis through chemotherapy gains our understanding of the DNA damage response in normal and tumor cells. In this context a large body of evidence indicates that nuclear cofactors targeting E2F1 seem to have a major impact on its tumor suppressor function. These new findings are discussed in the context of preclinical studies applying E2F1 overexpression in combination with genotoxic anticancer agents – called *chemogene* therapy, thereby providing new mechanistic links between the E2F1-induced apoptotic programming and advanced cancer phenotype.

1. Introduction

The E2F transcription factors have initially been identified as being critical in the control of cell cycle progression by regulating the timely expression of genes required for DNA synthesis at the G1/S phase boundary (DeGregori, 2002). E2Fs are a family of DNA binding proteins whose activity is intimately controlled through association with hypophosphorylated retinoblastoma protein RB and the pocket proteins p107 and p130. During cell cycle progression, D-type cyclin associated kinases initiate phosphorylation of RB family proteins, which results in the release of E2F and transactivation of E2F-regulated genes (Dyson, 1998). The E2F family consists of eight members (E2F1–E2F8) that are subdivided into two classes based on their transcriptional regulatory activities: the activator E2Fs (E2F1–E2F3a) that are potent transcriptional activators driving G0 cells to cycle, and the repressor E2Fs (E2F3b–E2F8) with weak activation potential that appear to be involved primarily in gene silencing of quiescent or differentiated cells.

The E2F proteins perform distinct, perhaps overlapping, functions in the control of cell cycle progression and have unique roles during development, tissue homeostasis and apoptosis (Stanelle and Pützer, 2006). During the course of tumor development cells sustain mutations that disrupt their normal growth control mechanisms. Mutations of the retinoblastoma tumor suppressor gene or components regulating the RB pathway have been identified in almost every human malignancy. This results in deregulated and hyperactive E2F in transformed cells leading to uncontrolled cell proliferation. However, E2Fs' role in determining cell fate is not restricted to their effects on cell cycle progression. Compelling evidence indicates that particularly E2F1 can also efficiently induce programmed cell death as part of an anti-tumorigenic safeguard mechanism that is critical in protecting the organism from malignant transformation and for suppression of tumor formation. Overexpression of E2F1 leads to apoptosis both *in vivo* and in tissue culture (Holmberg et al., 1998; Hsieh et al., 1997; Hunt et al., 1997; Phillips et al., 1997; Pierce et al., 1999; Qin et al., 1994; Shan and Lee, 1994). The apoptotic response to deregulated E2F was best demonstrated by the observation that RB-deficient mouse embryos show increased apoptosis, which is suppressed by the loss of E2F1 (Tsai et al., 1998). Furthermore, deletion of E2F1 in mice showed defects in thymocyte apoptosis with a higher resistance to death

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inducing stimuli, and increased tumor incidence (Field et al., 1996; Yamasaki et al., 1996).

One of the first signals recognized to induce E2F1 apoptotic activity was DNA damage (Blattner et al., 1999). The DNA damage signalling pathway is a highly conserved response to genotoxic stress (Zhou and Elledge, 2000). In mammalian cells, the pathway functions to protect cells from agents that induce cellular death or transformation, participating in DNA repair and checkpoint control leading to survival or apoptosis. In response to DNA damage, mammalian cells initiate a cascade of phosphorylation events mediated by two phosphatidylinositol-3-related kinases, ATM (ataxia telangiectasia mutated) and ATR (ATMRad3-related), which transduce signals to downstream targets and the checkpoint kinases Chk1 and Chk2 (Abraham et al., 2002; Banin et al., 1998; Canman and Lim, 1998; Shiloh, 2001). ATM is activated primarily by double-strand breaks induced by ionizing radiation, whereas ATR also responds to ultraviolet (UV) radiation or stalled replication forks (Osborn et al., 2002). The current model of the DNA damage response describes a linear progression beginning with sensors that convey the initial damage signal to mediators and transducers, which in turn transmit the signal to numerous effectors. It has been shown that treatment of cells with DNA damaging agents can induce endogenous E2F1, and aberrant expression of this transcription factor was found to increase the sensitivity of certain neoplastic cell types to apoptosis when treated combined with genotoxic agents (Meng et al., 1999; Polager and Ginsberg, 2003; Rödicker et al., 2001). A significant contribution to understanding of the physiological role of E2F1 in control of apoptosis that follows genotoxic stress came from *Drosophila* mutant flies (Moon et al., 2005), indicating that the role of E2F1 within individual developing wing discs exposed to γ-irradiation is extremely context dependent. Their and other data suggested that E2F1 protects non-proliferating cells while it sensitizes proliferating cells to γ-irradiation-induced cell death (Chen et al., 2009; Moon et al., 2005; Wikonkal et al., 2003). In normal cells, in the absence of DNA damage and in the presence of proper external signals for survival and growth, the apoptotic potential of E2F1 is suppressed. Conversely, when normal cells experience DNA damage or lack growth and survival signals, its apoptotic activity is unleashed. The induction of E2F1 by DNA damage occurs through several molecular mechanisms that affect the pRB–E2F1 interaction, E2F1 stability and/or the binding of E2F1 to promoters of specific E2F-regulated genes.

2. Activating E2Fs as responders to DNA damage-induced genotoxic stress

The E2F1 protein level is, like the tumor suppressor p53, upregulated in response to DNA damage (Blattner et al., 1999; Hofferer et al., 1999; Huang et al., 1997). This occurs at least in part through increased protein stability due to the phosphorylation of E2F1 at Serine 31 and 364 by the DNA damage sensors ATM, ATM- and Rad3-related protein (ATR), Chk1 and Chk2 (Lin et al., 2001; Stevens et al., 2003; Urist et al., 2004). Small interfering RNA (siRNA)-mediated knockdown of either Chk1 or Chk2, for example, was shown to prevent the induction of E2F1 protein in response to DNA damage (Urist et al., 2004). Because E2F1 also functions upstream of these kinases by transcriptionally regulating their expression, DNA damage triggers a positive-feedback loop between E2F1 and ATM/Chk2 (Berkovich and Ginsberg, 2003; Powers et al., 2004; Rogoff et al., 2002). Moreover, a recent study indicated that E2F1 is phosphorylated on Serine 403 after doxorubicin treatment (Real et al., 2010). E2F1 protein mutated at this site showed reduced efficacy to transactivate target promoters during DNA damage stress, but it remains to be determined which kinase is responsible for Ser403 phosphorylation and whether this has influence on E2F1s ability to induce apoptosis. Although phosphorylation alone leads to increased stability of E2F1 protein, further protein interactions are needed to compete with E2F1 protein degrading processes. In this context, the phosphoserine-binding protein 14-3-3τ seems to have outstanding significance (Wang et al., 2004). 14-3-3τ has high affinity to ATM-phosphorylated E2F1 during DNA damage, hence blocking its ubiquitination. Knockdown experiments demonstrated that 14-3-3τ and E2F1, but not E2F2 or E2F3, are critical for apoptosis induction by doxorubicin, and that 14-3-3τ is required for E2F1-mediated expression of proapoptotic targets such as p73, Apaf-1, and caspases in response to genotoxic stress. In addition, the apoptotic function of E2F1 upon DNA damage can be potentiated by posttranslational acetylation, which leads to enforced recruitment of the transcription factor to promoters of proapoptotic genes (Pediconi et al., 2003). This is mediated via binding of the stress inducible histone acetyl transferases P/CAF (p300/CREB-binding protein associated factor) and p300 to E2F1 (Galbiati et al., 2005; Ianari et al., 2004), and has been shown to affect E2F1 transcriptional activity towards target selection, resulting in selective induction of p73 for a favorable apoptotic response (Pediconi et al., 2003). But the increase in E2F1 protein levels is not only a reflection of enhanced protein stability. As recently reported, genotoxic drugs can induce the transcription of E2F1 mRNA as well (Carcagno et al., 2009). Therefore E2F1 target gene induction by DNA damaging agents might be the result of two well-differentiated effects, which depend on protein stabilization and *de novo* protein synthesis (Fig. 1).

Nevertheless, what seemed to be a transcriptional E2F1 feedback loop, turned out to be more complex. The Chauchereau group recently found that E2F3a is a substrate of the checkpoint kinases Chk1 and Chk2 (Martinez et al., 2010). Both E2F3 isoforms are identical except that the E2F3a protein has an additional 132 amino acids in its N terminus, which contains a chk motif that permits the protein to be phosphorylated by DNA damage. It is intriguing that neither Serine 124 in human E2F3a nor Serine 364 in human E2F1 are conserved in the mouse. One can only speculate that these chk motifs in the human E2Fs have been evolutionary adopted recently and constitute a mechanism for a rapid increase in protein levels. Their data revealed that E2F1 and E2F2 are transcriptionally induced upon DNA damage in an E2F3-dependent manner (Martinez et al., 2010), suggesting an interdependency between E2F1 to E2F3 in activating each other after genotoxic stress. On the basis of these results, it appears that upregulation of all three E2F transcription factors is part of the DNA damage response machinery. However, although it was recently shown that all activating E2Fs are able to transactivate promoter constructs of certain apoptotic target genes (Cassimere et al., 2009), a previous study reported that E2F3a could not induce apoptosis in cells deficient for E2F1, whereas E2F1 did not require E2F3 for the induction of cell death (Lazzerini Denchi and Helin, 2005). Such observations would support a model in which total E2F1 activity is the determinant of whether cells will undergo apoptosis (Lazzerini Denchi and Helin, 2005). This hypothesis is confirmed by other groups which associate apoptosis function of E2F1 with specific cofactors induced by DNA damage.

3. Role of RB in E2F1-mediated genotoxic stress responses – a challenge to the RB–E2F paradigm

The induction of E2F1s apoptotic activity by DNA damage affects its interaction with the pRB protein. This raises the question whether pRB counteracts E2F1s response upon DNA damage or both tumor suppressors cooperate to promote apoptosis. pRBs tumor suppressive activity is thought to be largely dependent upon its ability to directly bind members of the E2F family in a hypophosphorylated state and prevent them from promoting transcription of their target genes (Trimarchi and Lees, 2002). In general, this

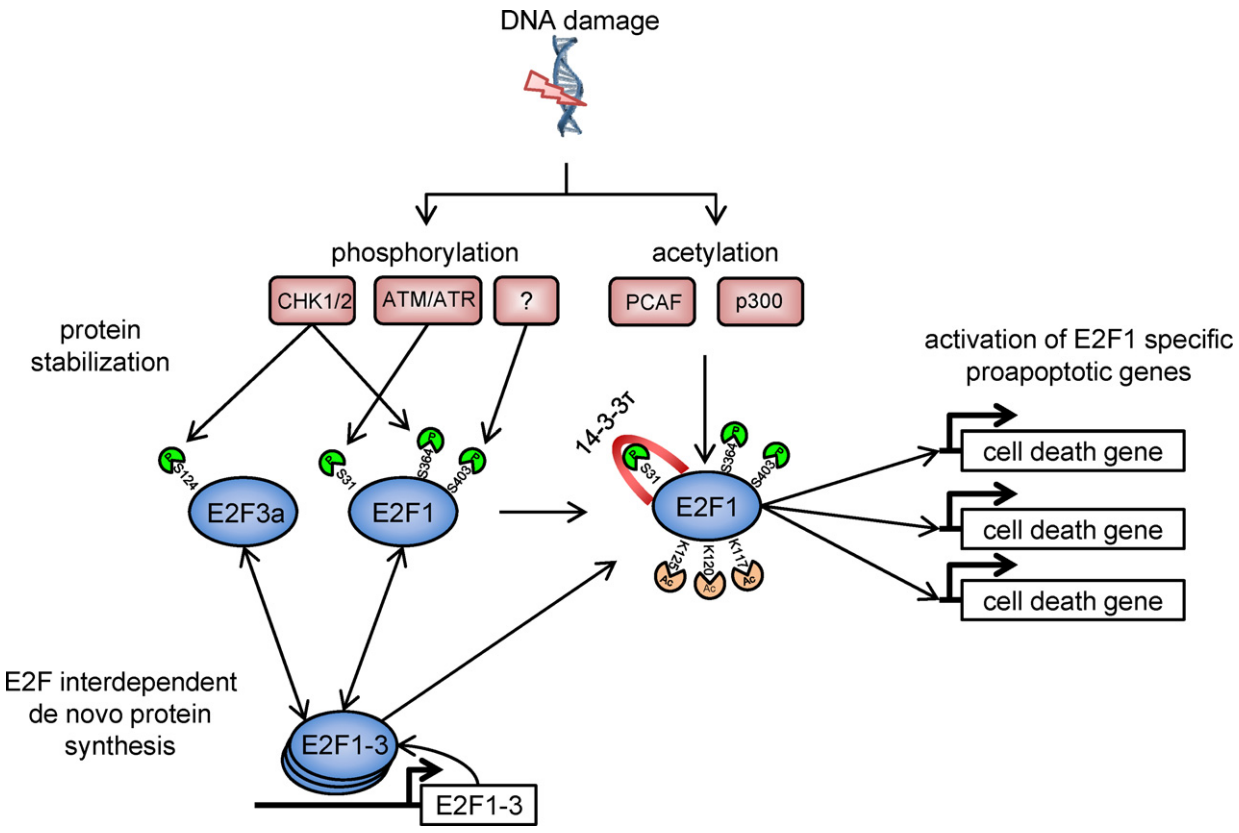

Fig. 1. Upregulation of activator E2F transcription factors is part of the DNA damage response machinery. Cytotoxic drugs stimulate DNA damage sensor kinases ATM/ATR and CHK1/2 which in turn phosphorylate E2F1 (Serine 31, 364) and E2F3a (Serine 124) causing rapid protein stabilization. 14-3-3τ proteins protect E2F1 from dephosphorylation. Promoter autoactivation of E2F1-3 leads to their de novo protein synthesis with the ultimate goal to maximize E2F1 protein levels. When E2F1 becomes acetylated by DNA damage sensitive histone acetyl transferases PCAF or p300 (Lysine 125, 120, 117), it can efficiently transactivate a subset of proapoptotic target genes. Hence, E2F1 is the unique executor of the death program in response to DNA damage inflicting drugs.

inhibition occurs through pRB binding with its pocket domain to sequences within E2Fs transactivation domain, and the resulting pRB–E2F complex recruits a number of transcriptional corepressors such as histone deacetylases (HDACs). In the case of E2F1, an additional C-terminal domain of pRB also binds the transcription factor (Dick and Dyson, 2003). This secondary binding site within pRB inhibits specifically E2F1-induced apoptosis and binding of E2F1 to this site is lost after DNA damage (Dick and Dyson, 2003). In particular, this domain becomes acetylated after DNA damage, resulting in E2F1 release without affecting the binding of pRB to other E2Fs (Markham et al., 2006). As a consequence of DNA damage-induced pRB acetylation, E2F1 can accomplish its tumor suppressor function by inducing the expression of apoptotic target genes (Fig. 2A, right). Apart from that, DNA damage induces not only the dephosphorylation of pRB at cyclin-dependent kinase (CDK) phosphorylation sites and binding of pRB to E2F1, but also pRB phosphorylation at Ser612 by Chk1/2 in an ATM-dependent manner (Inoue et al., 2007). Notably, this DNA damage specific phosphorylation is not associated with disruption of the pRB/E2F1 (E2F2 or E2F3) complex, suggesting that the widely assumed dogma that hyperphosphorylated pRB is functionally inactive and dephosphorylation restores pRB to the active state seems to lose validity after DNA damage. Ser612-phosphorylation resulted in pRB-dependent inhibition of E2F1-induced transcription of cell death genes such as p73, thereby efficiently counteracting E2F1s response to genotoxic stimuli (Fig. 2A, left) (Inoue et al., 2007).

In contrast, other studies provided the molecular basis for pRBs direct role in enhancing E2F1 apoptosis after DNA damage. Genotoxic stress induces pRB to participate in a transcriptionally active complex with E2F1 and the histone acetylase P/CAF that drives expression of proapoptotic genes like p73 and caspase 7 (Fig. 2B) (Ianari et al., 2009). While pRB knockdown experiments reduced transcription and dampened apoptosis upon chemotherapy *in vitro* and *in vivo*. At the same time, RB was shown to inhibit transcription of cell cycle related genes by formation of an RB–HDAC–E2F1 complex. These results are in accordance with an earlier study, suggesting that E2F1 is directed from cell cycle progression to apoptotic E2F target genes after DNA damage, and that PCAF activity is important for the potentiation of E2F1-dependent apoptotic responses (Pediconi et al., 2009).

Based on the above mentioned studies, pRB seems to have a dual role in response to DNA damage, acting both as E2F1 repressor and activator. Chk1/2 phosphorylation rather promotes E2F1-3 repression, whereas C-terminal pRB acetylation allows in theory E2F1-dependent apoptosis. On the contrary, pRB can permit apoptosis through direct binding of E2F1. Whether these post-translational modifications are required in physiological settings to enhance E2F1s transcriptional activity upon stress signals remains elusive. Yet, the current data clearly indicate that pRB plays a much more nuanced perhaps cell context-dependent role in the regulation of E2F1-induced apoptosis in damaged cells.

4. Cofactors regulating E2F1 DNA damage response signalling

4.1. Jab1

As the first proapoptotic cofactor of E2F1, Jab1 was identified. Jab1, originally found as a specificity factor for c-Jun and JunD transcription factors, interacts specifically with E2F1 via the marked box domain and mediates E2F1-induced apoptosis (Hallstrom and Nevins, 2003). This conserved region of E2F1 was previously shown to have unique proapoptotic activity that distinguishes E2F1 from

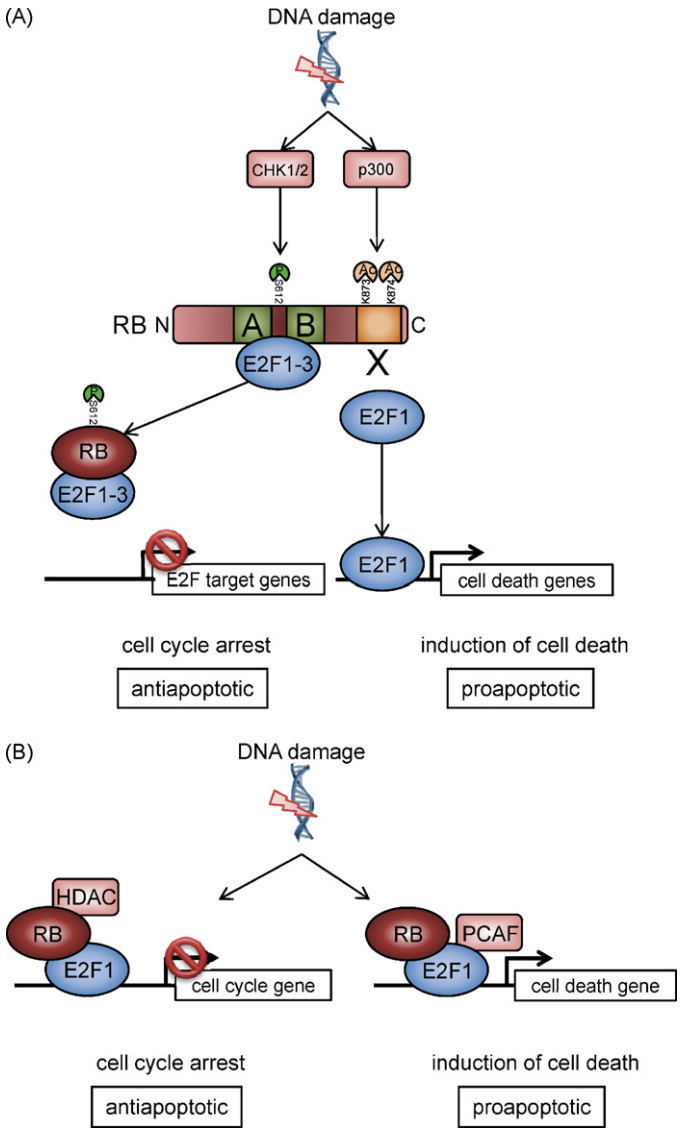

Fig. 2. The influence of RB on the E2F1-3 DNA damage response. Two models are under debate: (A) pRB acts as indirect regulator of E2F1-dependent apoptosis. Upon DNA damage pRB becomes phosphorylated at Serine 612 between the pocket domains A and B, leading to a stable pRB–E2F1–3 complex that inhibits E2F-dependent gene expression (left). Acetylation of the C-terminal E2F1 binding domain (orange shading) provokes the release of E2F1 (without affecting E2F2 or E2F3) and free E2F1 transactivates apoptotic target genes (right). (B) pRB directly stimulates E2F1-dependent apoptosis when cell are exposed to stress signals. The Rb-E2F1 complex is part of the active transcription machinery on proapoptotic E2F target genes and required for a maximal cell death response (right). Likewise, pRB acts as repressor of E2F1 on promoters of cell cycle genes by recruiting HDACs (left).

other E2Fs (Hallstrom and Nevins, 2003). Further investigations of how E2F1 specifically regulates antioncogenic apoptosis though other activating E2Fs such as E2F3a are able to bind certain proapoptotic E2F target gene promoters (Cassimere et al., 2009) pointed to the involvement of E2F1 specific cofactors that enhance the apoptotic gene expression pattern after DNA damage. Over the past years there has been a substantial increase in our understanding of the mechanistic control of E2F1 driven gene expression by cofactors that, like Jab1, bind E2F1 and thereby have a significant influence on its DNA damage response signalling.

4.2. BRIT1/MCPH1

One of these cofactors participating in E2F1-induced apoptosis upon cellular stress is BRCT-repeat inhibitor of hTERT expression/microcephalin (BRIT1/MCPH1), a breast cancer susceptibility gene 1 (BRCA1) C-terminal domain (BRCT) containing protein. There is clear evidence, that BRIT1/MCPH1 colocalizes with mediator of DNA damage checkpoint 1 (MDC1) and γ-H2AX in response to ionizing radiation, suggesting that it is required for the formation of DNA damage response foci (Lin et al., 2005; Rai et al., 2006; Xu et al., 2004). Strikingly, BRIT1/MCPH1 siRNA caused radio-resistant DNA synthesis and repression of BRAC1 and CHK1 at the transcriptional level via a mechanism that involves E2F1. Based on recent findings, MCPH1 cooperates with E2F1 to regulate genes involved in DNA repair, checkpoint and apoptosis such as RAD51, DDB2, TopBP1, p73 and caspases upon chemotherapy through complex formation on the promoters of these genes (Yang et al., 2008). This interaction depends on the amino terminus of E2F1. Additionally, E2F2 which is also upregulated in response to DNA damage (Martinez et al., 2010) is bound by MCPH1, even though E2F2s function in DNA damage is as yet unclear.

4.3. RRP1B

In an attempt to identify genes specifically regulated by E2F1 that mediate E2F1-induced cell death, Paik et al. (2010) identified the ribosomal RNA processing 1 homolog B (RRP1B) as target of E2F1 that is not activated by other E2F family members. RRP1B is important for apoptosis regulation by DNA damage and E2F1 overexpression. Because this activity is mediated in part by physical interaction between RRP1B and E2F1 on its own and other E2F1 target gene promoters, RRP1B acts apparently as coactivator to prime cells for E2F1-dependent killing after genotoxic stimuli. Whereas the DNA binding domain of E2F1 seems to be critical for this interaction, the mechanism by which RRP1B controls E2F1s transcriptional activity is unknown. Since RRP1B does not contain any known DNA binding or transactivation motif, the possibility exists that activation occurs by displacing negative cofactors from the DNA binding domain.

4.4. HCF-1

The host cell factor 1 (HCF-1) originally discovered as a cellular coactivator of herpes simplex virus (HSV) transcription is a heterodimeric complex of N- and C-terminal subunits. The N-terminal subunit recognizes and binds to a short tetrapeptide HCF-1 binding motif (HBM) with numerous transcription factors. In particular, E2F1 associates through a short DHQY sequence with HCF-1 next to the cyclin binding motif (CBM) within the cyclin A/CDK2 binding domain. When bound to E2F1, HCF-1 displays coactivator properties by selective recruitment of histone-modifying activities, including the mixed-lineage leukemia (MLL) and Set-1 histone H3 lysine 4 methyltransferases to E2F1-responsive promoters, thereby triggering histone methylation and transcriptional activation (Tyagi et al., 2007). Consistent with a function of HCF-1 in E2F1-mediated apoptosis in response to DNA damage induction, E2F1, HCF-1 and MLL are recruited to proapoptotic E2F target promoters upon doxorubicin treatment and activate transcription (Tyagi and Herr, 2009). Inhibition of either HCF-1 or H3K4 methyltransferase activity by depletion experiments has confirmed their function for E2F1s ability to respond to DNA damage.

4.5. DP proteins

Further insight into the regulation of E2F1 activity during DNA damage was gained by the La Thangue group (Milton et al., 2006), suggesting that the interaction between 14-3-3ε and the DP family subunit DP-3 is of functional importance. DP proteins, DP-1 and DP-2 (equivalent to murine DP-3) were shown to be essential for E2Fs DNA binding activity by forming E2F/DP heterodimers that bind

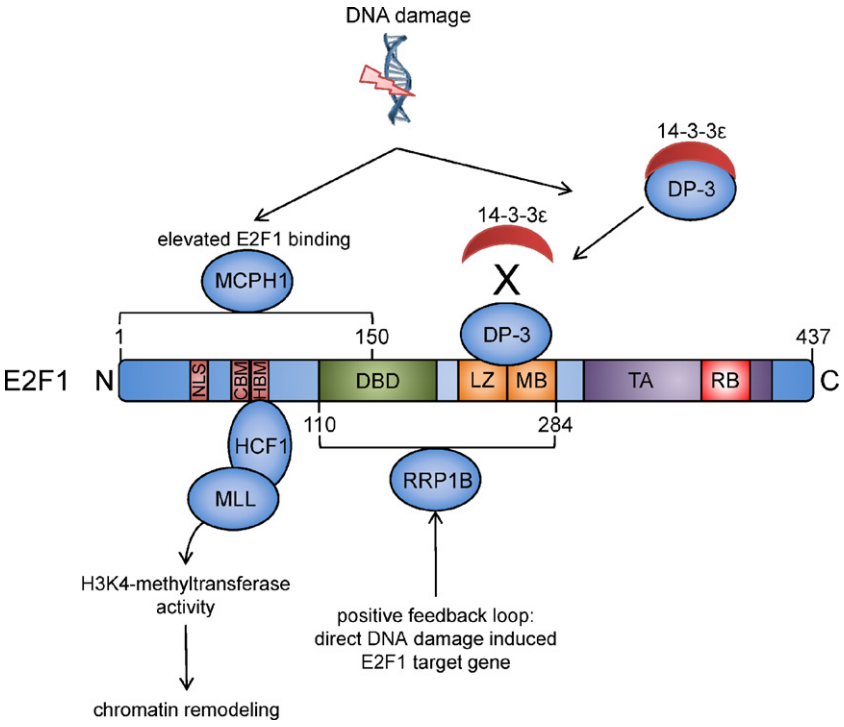

Fig. 3. Nuclear cofactors are essential for E2F1s tumor suppressor function. Upon DNA damage, MCPH1 binds the N-terminal region of E2F1 and positively regulates its transcriptional activity. The HCF1 binding motif HBM is in close proximity to the cyclin binding motif (CBM). HCF1 recruits H3K4 histone methyl transferases to activate gene transcription. RRP1B is a direct E2F1 target gene induced after DNA damage that physically interacts with E2F1s DNA binding domain (DBD), leucine zipper domain (LZ), and marked box domain (MB). This binding is required for E2F1s ability to induce apoptosis. E2F transcription factors dimerize with DP proteins to bind DNA. The E2F1/DP-3 complex is induced in response to stress signals by disruption of the binding of 14-3-3ε to DP-3. NLS, nuclear localization signal; TA, transactivation domain; RB, pocket protein binding domain.

to and regulate E2F target genes (Trimarchi and Lees, 2002). They found that DP-3 physically interacts with 14-3-3ε in intact cells (Milton et al., 2006). Disruption of the 14-3-3ε/DP-3 complex either by DNA damage or siRNA-mediated 14-3-3ε knockdown caused elevated apoptosis, which correlated with increased levels of DNA damage-responsive DP-3. These results implicated DP-3 as part of the activated E2F1 transcription complex during DNA damage that specifically triggers apoptosis. The set of nuclear coregulators supporting the E2F1-induced antioncogenic DNA damage response is summarized in Fig. 3.

4.6. Additional interactions

Apart from drug-induced factors directly interacting with E2F1 to cooperate in cell death, another mechanism of genotoxic agents to trigger E2F1-induced apoptosis is the disruption of E2F-repressive complexes from proapoptotic target gene promoters. It is well known that C-EBPα is part of the E2F4–p107 complex antagonizing E2F1s transcriptional activity (Chen et al., 1996; Timchenko et al., 1999). It was suggested that treatment of cells with DNA damaging agents induces nuclear export of C-EBPα and blocking of this export abolished drug-induced p73 activation (Marabese et al., 2003). Moreover, oncogenes like adenoviral E1A protein lead to an activation of E2F1s proapoptotic machinery (Ianari et al., 2009). A recent study showed that upregulation of E2F1 and sensitization to chemotherapy-induced death requires the region of E1A which binds p400 (Helgason et al., 2010). The authors substantiated a direct protein–protein interaction between E2F1 and p400. Knockdown of p400 enables E2F1 induction and sensitizes cells expressing an E1A mutant lacking p400 binding to chemotherapy (Helgason et al., 2010). Although the exact molecular mechanism remains to be elucidated, E1A may sequester p400 away from E2F1 enabling its function to prime for genotoxic agents.

5. Multiple pathways leading to E2F1 driven apoptosis

In recent years, extensive studies have been made to answer the question how E2F1 initiates apoptosis. Here, a large number of proapoptotic target genes have been shown to be E2F1 regulated and it is most likely that many still remain to be identified. Although E2F1 is mainly believed to be an activator of gene expression, it became apparent that this protein also immediately represses transcription of genes directly involved in cell survival. In fact, it was shown that E2F1-3 can function as activators and repressors depending on the cell context (Chong et al., 2009). E2F1s signalling to apoptosis remains complex since promoter binding, direct protein–protein interactions and modulation of posttranslational RNA modification contribute to its ability to trigger a variety of cell death pathways as discussed below.

5.1. E2F1 activates the p53 family

Commonly, mechanisms of apoptosis induced by the activation of E2F1 either by overexpression or as response upon DNA damage inflicting drugs are classified as being p53-dependent or p53-independent. The first described mechanism by which E2F1 induces apoptosis is the activation of p53 due to protein stabilization. While E2F1 does not bind to the p53 promoter, transactivation of p14ARF leads to p53 accumulation, which in turn causes apoptosis by activating p53 target genes (Bates et al., 1998; Hiebert et al., 1995). Although E2F1 was the first family member being discovered to initiate p53-dependent apoptosis, all activator E2Fs are able to directly bind and stabilize p53 via their cyclin A binding domain. Cyclin A normally prevents E2F1 from interacting and cooperating with p53. However, in response to DNA damage, cyclin A levels decrease, with a concomitant increase in E2F1–p53 complex formation (Hsieh et al., 2002). In addition, E2F1 triggers p53 protein stabilization during DNA damage through transcriptional

activation of ATM, CHK1 and CHK2 leading to phosphorylation of p53 (Berkovich and Ginsberg, 2003; Yang et al., 2008). Of note, the nutrient energy sensor AMP kinase α 2 (AMPKα2), identified as an E2F1 target gene in p53-positive cells, has been implicated in E2F1s apoptotic activity (Hallstrom et al., 2008). Future research has to clarify whether this signalling requires functional p53 because AMPKα2 phosphorylates p53 (Jones et al., 2005). Others reports have shown that E2F1 guided p53 activation occurs via pathways in addition to protein stabilization (Polager and Ginsberg, 2009). E2F1 can, for example, upregulate the expression of p53 apoptotic cofactors, such as ASPP1, ASPP2, JMY and TP53INP1, which have been shown to direct p53 apoptotic response through whether enhancing p53 interaction with proapoptotic targets or promoting p53 phosphorylation (Hershko et al., 2005).

Almost at the same time when the role of E2F1 as a potent activator of p53 was established several studies in tissue culture and transgenic mice models demonstrated that E2F1 can induce cell death also independent of p53 (Hsieh et al., 1997; Phillips et al., 1997). To date, we know that this is due to the fact that E2F1 transactivates a large number of proapoptotic genes. Noteworthy is the finding that E2F1 and p53 share an overlapping but not identical subset of target genes like APAF1, SIVA, NOXA or PUMA (Fortin et al., 2004; Furukawa et al., 2002; Hershko and Ginsberg, 2004). Most importantly E2F1 was shown to directly activate transcription of the p53 homolog p73 (Irwin et al., 2000; Stiewe and Pützer, 2000; Vilgelm et al., 2008), which in turn stimulates gene expression patterns that again partly overlap with those of p53 and E2F1. This pattern of regulation constitutes a modified feedforward loop (Polager and Ginsberg, 2009) that can be understood as a network whereby p53, p73 and E2F1 cooperate to activate the same set of genes to subsequently gain a maximum induction of cell death. On the other hand, redundancy of p53, p73 and E2F1 in gene activation in addition to their ability to upregulate a particular proapoptotic gene set represents a failsafe tumor defense mechanism.

5.2. *E2F1 is a strong inducer of the mitochondrial death pathway via a plethora of mediators*

Elucidation of death pathways triggered by E2F1 revealed a large number of directly regulated proapoptotic genes (Fig. 4). These findings underscore the role of E2F1 as a key mediator of mitochondrial outer membrane permeabilization by positively regulating the expression of virtually all known BH3-only proteins (BID, BIK, BIM, BNIP3, HRK, NOXA and PUMA) (Cao et al., 2004; Hershko and Ginsberg, 2004; Real et al., 2006; Stanelle et al., 2002) that trigger complex formation of proapoptotic BH1-3 proteins with subsequent cytochrome *c* release (Zhang et al., 2007). Moreover, apoptosis inducing BH1-3 proteins like BOK are upregulated by E2F1 (Rodriguez et al., 2006). Furthermore, E2F1 downregulates expression of the anti-apoptotic Bcl-2 family member Mcl-1 (Croxton et al., 2002). It can also affect the expression of Bcl-2, but the exact nature of this effect is under debate. Although some studies showed that E2F1 inhibits Bcl-2 expression, others demonstrated increased protein levels (Eischen et al., 2001; Gomez-Manzano et al., 2001b). Since activation of death pathways at multiple levels is a common feature of E2F1, it is not surprising that E2F1 driven apoptosis is carried out via transcriptional induction of several caspases, including caspase-3, -7, -8, -9 (Cao et al., 2004; Nahle et al., 2002), and Apaf-1 (Cao et al., 2004; Xie et al., 2006). E2F1 also blocks caspase inhibition by direct transactivation of Smac/DIABLO (Xie et al., 2006).

In addition, there is growing evidence that many immediate E2F1 target genes indirectly trigger mitochondrial apoptosis. These include FOXO3 (Nowak et al., 2007) belonging to the forkhead family of transcription factors, which has been shown to drive apoptosis via transcriptional activation of BIM, NOXA and PUMA (Dijkers et al., 2000; Obexer et al., 2007; You et al., 2006a). Similar to E2F1, FOXO3 triggers cell death by binding to p53 (You et al., 2006b). FOXO3 is upregulated by chemotherapy and mediates E2F1-induced chemosensitization of cancer cells (Engelmann et al., 2010; Obexer et al., 2009). Thus, the E2F1–FOXO3 axis represents a novel feedforward loop through which FOXO3 fortifies apoptosis by activating in part the same cell death pathways as E2F1. Although E2F1 does not directly transactivate BAX, a number of novel E2F1-regulated genes are able to enforce BAX expression next to p73. The transcription factor E1AF was shown to mediate sensitivity of cancer cells to etoposide-induced cell death in response to E2F1 upregulation (Wei et al., 2008). Mechanistically, E1AF physically interacts with the BAX promoter, leading to caspase-dependent cell killing in conjunction with chemotherapy (Liu et al., 2008; Wei et al., 2008). Other inducers of the mitochondrial death pathway whose expression is controlled by E2F1 are the non-classic serine protease inhibitor maspin (SERPINB5) (Ben Shachar et al., 2010), a known target of p53, and the Krüppel-like transcription factor KLF10 (Engelmann et al., 2010). KLF10 has been shown to promote mitochondrial apoptosis induced by oxidative stress (Ribeiro et al., 1999), by homoharringtonine or by bortezomib involving Bax upregulation and caspase-3 activation (Jin et al., 2007). Both maspin and KLF10 tumor suppressors have been demonstrated to act as effectors of E2F1-induced sensitization of tumor cells to genotoxic agents (Engelmann et al., 2010; Ben Shachar et al., 2010).

Some clarity has been shed on the requirement of the ASK1-p38 MAPK pathway for E2F1-induced apoptosis. E2F1 modulates the activity of the p38 MAPK pathway through upregulation of p38 MAPK phosphorylation (Hershko et al., 2006). 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 by direct promoter binding (Kherrouche et al., 2006). The relevance of the ASK1–p38 connection for E2F1-mediated apoptosis is also evidenced by data demonstrating that E2F1 regulates the activity of the p38 signalling inhibitor Wip1. Knockdown of Wip1 has been shown to enhance E2F1-dependent apoptosis (Hershko et al., 2006). Exogenous expression of wild-type ASK1 or constitutively active ASK1 induces death of various cells mainly through mitochondria dependent caspase activation (Hatai et al., 2000). Overall it is evident that activation of the mitochondrial pathway is a key node within the E2F1-induced cell death network, emphasizing that redundancy seems to have outstanding importance during the evolutionary development of E2F1 apoptosis.

5.3. *Inhibition of survival signalling*

Another general mechanism by which E2F1 sensitizes cells to apoptosis is inhibition of anti-apoptotic signalling. Activation of tumor necrosis factor receptor (TNFR) in response to TNF results in the stimulation of NF-κB via TRAF2, which contributes to the inhibition of cell death (Chen and Goeddel, 2002; McConkey and Zhu, 2008). E2F1 can downregulate TRAF2 protein levels, thereby inhibiting anti-apoptotic NF-κB signalling (Phillips et al., 1999). The direct E2F1 target SIVA (Fortin et al., 2004) seems to mediate this downregulation, since it promotes TRAF2 polyubiquitination and protein degradation (Gudi et al., 2009). Moreover, E2F1 inhibits DNA binding activity of NF-κB by interacting with its subunit p65 (Tanaka et al., 2002). Another example for its interference with cell survival signals is the inhibition of heat shock proteins. Unfolded protein response-mediated survival or cell death is regulated by the balance between endoplasmic reticulum (ER) chaperones GRP78 and Gadd153. E2F1 downregulates expression

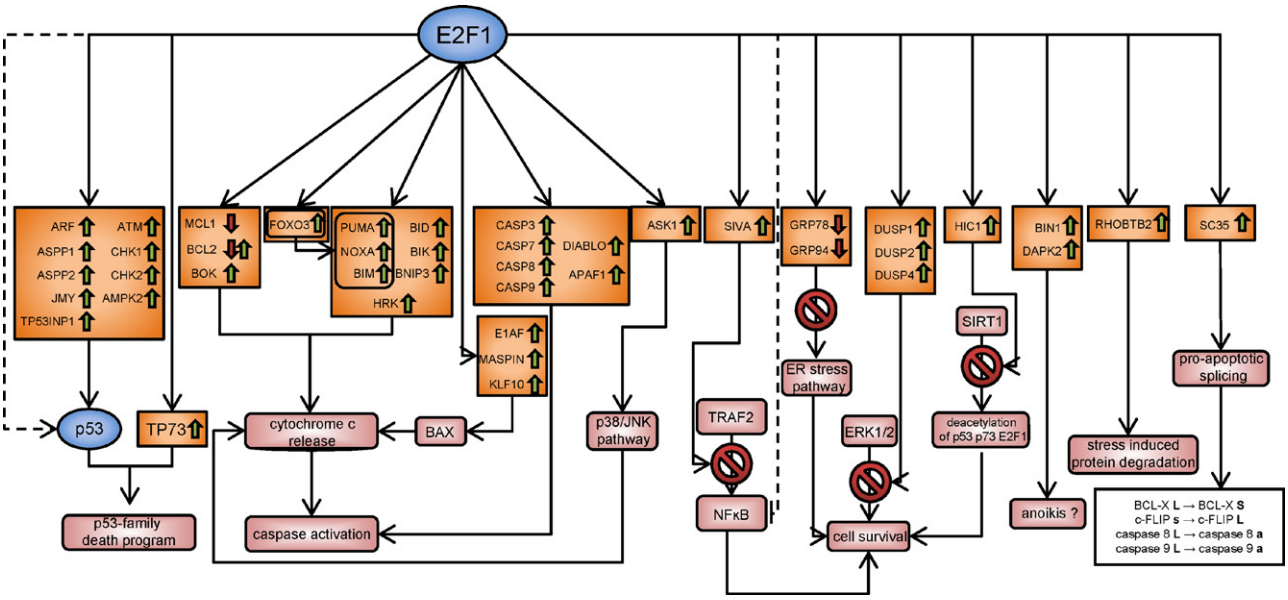

Fig. 4. Roadmap for E2F1 apoptotic signalling. E2F1 stimulates the p53 family related death program by directly and indirectly influencing p53 stability, or through transcriptional activation of TP73. E2F1 induces a variety of effector proteins, which in turn trigger cytochrom *c* release and caspase activation. Proapoptotic expression patterns of E2F1 and FOXO3 partly overlap. In addition, E2F1 disrupts cell survival signalling by suppressing NFκB, ER stress chaperons, ERK signalling, and inhibition of p53, p73 or E2F acetylation. Furthermore, E2F1 has also been linked to anoikis and stress induced protein degradation, and alters gene expression by triggering proapoptotic splicing. Orange boxes contain E2F1 target genes. Arrows indicate E2F1-induced up- (green) or downregulation (red). Dotted lines denote protein–protein interactions.

of GRP78 (also known as BIP) (Racek et al., 2008) normally protecting cells from death induced by disturbance of ER homeostasis (Miyake et al., 2000) by physical interaction with the ER stress response element of the GRP78 promoter to displace positive regulatory proteins Sp1 and TFII-I. Moreover, expression of the 94 kD glucose-regulated protein (GRP94) ER chaperone shown to be upregulated in human esophageal cancers was inhibited by E2F1 (Li et al., 2006; Wang et al., 2005). From these data it is evident that E2F1-mediated apoptosis involves ER-related death pathways. The MAPK cascade, one of the most predominant pathways for cell growth and proliferation is negatively regulated by dual specificity phosphatases (DUSP). Yin and colleagues found that E2F1 is able to inhibit MAPK signalling in response to chemotherapeutic drugs triggering the formation of ROS. Oxidative stress induces transcriptional upregulation of DUSP1, DUSP2 and DUSP4 in a strictly E2F1-dependent manner, which in turn dephosphorylate ERK. Knockdown experiments revealed that E2F1-mediated shut-down of ERK signalling is required for ROS-induced cell death (Wang et al., 2007b,c; Wu et al., 2007). SIRT1 is a NAD^+-dependent class III deacetylase regulating cell survival by binding and antagonizing transcriptional activities of p53, p73 and E2F1. E2F1 can upregulate the expression of SIRT1 upon DNA damage constituting a potential oncogenic negative feedback loop. The physiological relevance of these interactions is highlighted by an increased apoptotic activity of E2F1, p53 and p73 following SIRT1 inactivation (Dai et al., 2007; Vaziri et al., 2001; Wang et al., 2006). But recent studies showed that DNA damaging agents counteract SIRT1-related tumor suppressor deacetylation in two respects: First, the hypermethylated in cancer 1 (HIC1) gene, which is transcriptionally activated by E2F1 upon chemotherapy (Jenal et al., 2009), encodes a zinc finger transcriptional repressor that cooperates with p53 to suppress cancer development by antagonizing SIRT1 function in DNA damage response (Chen et al., 2005). Secondly, apoptotic DNA damage decreases NAD^+ and inhibits SIRT1 activity, thereby favoring the assembly of transcriptionally active PCAF/E2F1 complex onto the P1p73 promoter and apoptosis irrespective of the p53 status (Pediconi et al., 2009). This is an example how chemotherapeutics cooperate with E2F1 to induce cell death via two distinct mechanisms.

5.4. Non-canonical E2F1-induced cell death pathways

The role of E2F1 as an essential activator of classical apoptosis pathways is well-established. Recent evidence suggests that E2F1 modulates cell fate at least in part also via non-canonical cell death pathways. It was found that BIN1, originally identified as a c-MYC-interacting tumor suppressor, is directly regulated by E2F1 particularly after DNA damage (Cassimere et al., 2009). The established link between E2F1 and BIN1 might represent a pathway through which DNA damage-induced death is activated in cancer cells regardless of p53, p73 and caspases. But one is still groping in the dark when identifying the mechanism underlying apoptosis associated with the stimulation of BIN1. It is conceivable that the E2F1–BIN1 axis mediates anoikis since BIN1 was implicated in cell polarity signalling and tissue remodeling of different organisms (Prendergast et al., 2009). Another study that also links E2F1 to anoikis showed that death-associated protein kinase 2 (DAPK2) whose downregulation is required for β-catenin induced anoikis resistance of malignant epithelial cells (Li et al., 2009) is transcriptionally activated by E2F1 (Britschgi et al., 2008). Moreover, E2F1 provokes protein degradation as response to genotoxic stress via upregulation of RhoBTB2 (Freeman et al., 2008), although the mechanism beyond this observation remains elusive. Considering that a remarkable number of transcripts encoding proteins involved in the apoptotic pathway are subject to alternative splicing, it was unsurprising that E2F1 is also capable of controlling pre-mRNA processing events to execute apoptosis. In response to DNA damage, E2F1 switches genes including caspase-8 and -9, Bcl-X, and c-FLIP towards proapoptotic isoforms through the upregulation of splicing factor SC35 (Merdzhanova et al., 2008). Together with the ability to induces cap methylation essential for efficient gene expression (Cole and Cowling, 2009), these observations suggest that an important and hitherto unrecognized feature of E2F1 is the regulation of post-transcriptional processes to contribute to the induction of cell death.

An intriguing addition was made by latest reports, indicating that small non-coding RNAs (microRNAs or miRNAs) that negatively regulate eukaryotic gene expression at the post-transcriptional level are activated by E2F1-3a, and can in turn,

inhibit mRNA translation of all three activator E2Fs by pairing with complementary nucleotide sequence in their 3′-untranslated regions (Emmrich and Pützer, 2010). Some of the E2F1-regulated miRNAs have been implicated in the induction of apoptosis by antagonizing the expression of prosurvival genes and/or cooperate with p53 in the integration of apoptotic signals. Interestingly, miR-449a/b, recently discovered as direct transcriptional targets of E2F1 (Lize et al., 2010; Yang et al., 2009), were shown to diminish the SIRT1 deacetylase leading to p53 hyperacetylation and increased apoptosis after DNA damage (Lize et al., 2010). In addition, the use of miRNAs to indirectly target potential survival factors provides another general mechanism that enables E2F1 to modulate chemoresistance. *Vice versa*, E2F1-induced miRNAs of the miR-449 family inhibited E2F1 itself, thereby establishing a negative feedback loop that may be important for allowing mutual regulation of major DNA damage-responsive gene regulators. Given the rapid pace in the discovery of miRNA-E2F interactions, this all points to regulatory networks of high complexity that might ensure the tight control of E2F activities in a context-dependent manner.

6. Is E2F1 itself an anticancer agent?

6.1. E2F1 chemogene therapy approaches to overcome resistance to conventional anticancer treatment

A critical determinant of the efficacy of anticancer therapies is the ability of cancer cells to undergo apoptosis in response to DNA damaging agents (Qiao and Wong, 2009). The success of genotoxic treatments is attributed, at least in part, to the fact that DNA damage induces cell death more readily in cancer cells than in normal cells. The molecular mechanism(s) underlying the enhanced sensitivity of cancer cells to drug-induced apoptosis are not fully understood. As E2F1, which is frequently upregulated in human cancer cells, is inducible by apoptotic DNA damage, it appears here of particular significance. Therefore, many preclinical studies have attempted to apply E2F1 overexpression as anticancer therapy. The effect of E2F1 overexpression alone and in combination with chemotherapeutic drugs – called *chemogene* – on tumor cells has been evaluated in almost every common type of human cancer, including glioma, melanoma, esophageal cancer, breast- and ovarian carcinoma, head and neck squamous cell cancer, gastric cancer, pancreatic carcinoma, fibrosarcoma, osteosarcoma, leukemia and non-small-cell lung cancer (Banerjee et al., 1998; Ben Shachar et al., 2010; Dong et al., 1999, 2002; Elliott et al., 2002a,b; Engelmann et al., 2010; Fueyo et al., 1998; Gomez-Manzano et al., 2001a; Hunt et al., 1997; Kuhn et al., 2002; Liu et al., 1999; Nguyen et al., 2005; Nip et al., 1997; Parr et al., 1997; Pruschy et al., 1999; Rödicker et al., 2001; Yang et al., 1999, 2001). These studies have shown that apoptosis induction by the combination treatments resulted in increased responsiveness of tumor cells to chemotherapy while relatively sparing normal tissues. They also indicated that the mechanism of primary action of the used cytotoxic agents did not affect E2F1s ability to chemosensitize cancer cells. A variety of different genotoxic anticancer agents, including topoisomerase inhibitors (etoposide, doxorubicin, SN38, ICRF-193), alkylating agents (BCNU, temozolomide), antimetabolites (gemcitabine, fluorouracil), cross-linker (cisplatin), and antimitotic agents (roscovitine, vinblastine, paclitaxel) cooperated with E2F1 overexpression. Hence, it was not an unexpected finding that E2F1 also had activity as radiosensitizer (Pruschy et al., 1999; Nguyen et al., 2005). In sum, these experiments indicate a universal role of E2F1 in mediating and enhancing apoptotic signalling upon treatment with cytotoxic stressors in cancer cells.

Considering the potential impact of cofactors on E2F1's apoptotic response to genotoxic agents in a physiological context, recent data showed that RRP1B is involved in suppression of metastasis and a gene expression profile obtained following its overexpression predicted survival in breast cancers (Crawford et al., 2007). Thus, it is possible that increased patient survival seen in breast cancers with high RRP1B expression may be due to increased responsiveness to genotoxic therapy. Similar to RRP1B, a function of the E2F1 cofactor MCPH1 as a tumor suppressor has been suggested by association of genomic instability and metastasis with decreased levels of MCPH1 in human cancer (Rai et al., 2006). Given the crucial role of E2F1 in the chemotherapeutic response, decreased expression levels of E2F1 cofactors like MCPH1 or RRP1B in cancers might dampen the apoptotic response to chemotherapy owing to lesser induction of crucial E2F1 target genes preventing cancer progression.

In addition, several studies have shown that pharmacological modulation of endogenous E2F1 is an efficient strategy to sensitize tumor cells to DNA damaging agents. Recently, small-molecule inhibitors of MDM2 that impair p53 function by binding to the tumor suppressor protein, the nutlins, have been developed to reactivate p53 signalling in malignant cells with functional p53 by antagonizing the MDM2–p53 interaction (Vassilev et al., 2004). Nutlin-3a also inhibits E2F1s interaction with MDM2 in p53-mutant cancer cells, leading to increased E2F1-dependent upregulation of p73 and NOXA and apoptosis following DNA damage (Ambrosini et al., 2007; Kitagawa et al., 2008). This suggests that small-molecule inhibitors of E2F1 antagonists like nutlin can activate E2F1 for apoptosis, providing an extended chemotherapeutic option in human cancers with mutated p53.

However, irrespective of a potential benefit of increasing E2F1 activity to modulate tumor cell survival and chemoresistance regardless of the p53 status, newer reports teach us that this might be a double-edged sword. On one side, an investigation with 183 breast cancer patients who had undergone surgical resection revealed that the E2F1-positive group had less tumor recurrences, lymph node metastases during follow-up, and distant metastases than the E2F1-negative group (Kwon et al., 2010). This is in line with clinical surveys associating E2F1 expression with improved survival in patients treated with adjuvant chemotherapy for gastric cancer and colon cancer, underscoring the role of E2F1 as an endogenous chemosensitizer in cancer patients and potentially prognostic or predictive factor for clinical outcome and therapeutic results (Belvedere et al., 2004; Lee et al., 2008). Conversely, E2F1 expression was frequently associated with high grade tumors, unfavorable patient survival prognosis and tumor progression (Banerjee et al., 2000; Eymin et al., 2001; Gorgoulis et al., 2002; Han et al., 2003; Iwamoto et al., 2004; Salon et al., 2007; Yamazaki et al., 2003). Most strikingly, Lee et al. (2010) showed that high expression of E2F1 and its associated target genes predict superficial to invasive progression of bladder tumors in cancer patients. Progression of superficial tumors to invasive tumors was significantly higher in the E2F1-high group than in the E2F1-low group. Together with the finding that E2F binding sites are significantly enriched in promoters of genes whose expression is strongly associated with invasive tumors, these data suggest that E2F1 expression is functionally associated with invasive tumor development (Lee et al., 2010). Even though in those cases E2F1 expression in conjunction with chemotherapy was not examined, it is a known fact that cancers of advanced stage are commonly resistant against chemotherapy. Therefore, one might speculate that stimuli like DNA damage or oncogenic stress are required for an efficient tumor suppressor function of E2F1, and that its overexpression *in vivo* could be rather oncogenic than anti-neoplastic when death pathways are mutated. This notion was substantiated by a new transcriptome profiling approach, primarily focusing on the discovery of genes involved in a cooperative perhaps synergistic apoptosis response between E2F1 and chemotherapeutic drugs in p53-deficient tumor cells (Engelmann et al., 2010). While these experiments revealed upregulation of known and hitherto unknown E2F1 death genes, it

also uncovered unexpected risks of *chemogene* therapy for cancer patients. Importantly, the microarray data demonstrated a synergism in the upregulation of genes that are related to cell survival and tumor progression. Based on these results, it is conceivable that DNA damaging agents also promote oncogene activation in the context of E2F1 signalling. This assumption was further supported by the observation that conditional E2F1 activation in transgenic mice targeted to the testes, which results in the activation of E2F1 target genes and p53-independent apoptosis (testicular atrophy), caused also premalignant changes resembling carcinoma *in situ* in humans (Agger et al., 2005). Although the ability of genotoxic compounds to transcriptionally induce oncogenes in association with aberrant E2F1 activity did not affect the net outcome of enhanced killing of tumor cells *in vitro*, this potentially carcinogenic side effect might reduce efficacy of anticancer therapy in patients and favor tumor recurrence or progression following chemotherapy (Fig. 5A). Even more important, considering E2F1s natural prosurvival function in non-proliferating cells, this could eventually lead to tumor formation in normal tissues.

6.2. Evasion of tumor cells from E2F1-induced apoptosis as a mechanism of drug resistance

Although E2F1 is a versatile and very potent tumor cell death inducer, E2F1 does not always induce cell death. Therefore, increased understanding of the molecular mechanisms leading to resistance to E2F1-induced apoptosis by chemotherapy is a premise to improve current anticancer therapies, since anti-apoptotic negative feedback loops are important ways for tumor evasion (Fig. 5B).

It has been shown that the E2F1 inducible E2F7 and E2F8 proteins cooperate to repress E2F1-dependent apoptosis as a mechanism for cell survival (Li et al., 2008; Moon and Dyson, 2008). Moreover they counteract the cellular DNA damage response. E2F7 and E2F8 are induced in cells treated with DNA damaging agents and bind to the promoters of certain E2F-responsive genes, most notably that of the E2F1 gene, in which E2F7 and E2F8 coexist in a DNA binding complex (Zalmas et al., 2008). As a consequence, E2F7 and E2F8 inhibit E2F target genes, and reducing the level of each subunit results in an increase in E2F1 expression and activity. Thus E2F7 and E2F8 influence the ability of cells to undergo a DNA damage response.

An intriguing body of experimental data indicates that oncogenic PI3K–AKT signalling opposes E2F1 tumor suppressor function (Hallstrom et al., 2008; Hallstrom and Nevins, 2003). AKT inhibits E2F1-induced apoptosis indirectly through phosphorylating of DNA topoisomerase IIβ binding protein 1 (TopBP1) (Liu et al., 2006). Through its BRCT domain, TopBP1 interacts with and represses exclusively E2F1 but not E2F2 or E2F3 by recruiting Brg1/Brm, a component of the SWI/SNF chromatin-remodeling complex, to E2F1-responsive promoters, such as p73, p14ARF (Liu et al., 2004). This regulation of E2F1 transcriptional activity is crucial in the control of E2F1-induced apoptosis during normal cell growth and DNA damage and occurs independent of pRB. The Nevins' group further analyzed the ability of growth-factor-activated PI3K signalling to directly regulate E2F1 transcriptional output and apoptosis induction and identified a subset of E2F1 proapoptotic targets that are specifically blocked by a serum-activated PI3K-AKT signalling, while E2F1s genes involved in the proliferative program were not affected (Hallstrom et al., 2008). This implicates that oncogenic signalling acts as inhibitor of E2F1's apoptotic response in tumor cells. Reduced expression of genes belonging to the E2F1 apoptotic program in breast and ovarian cancer patients coincided with significantly poorer survival outcomes, emphasizing the importance of these events for establishing resistance to E2F1-induced apoptosis linked with an advanced type of cancer. Similarly, epidermal growth factor receptor (EGFR) signalling has

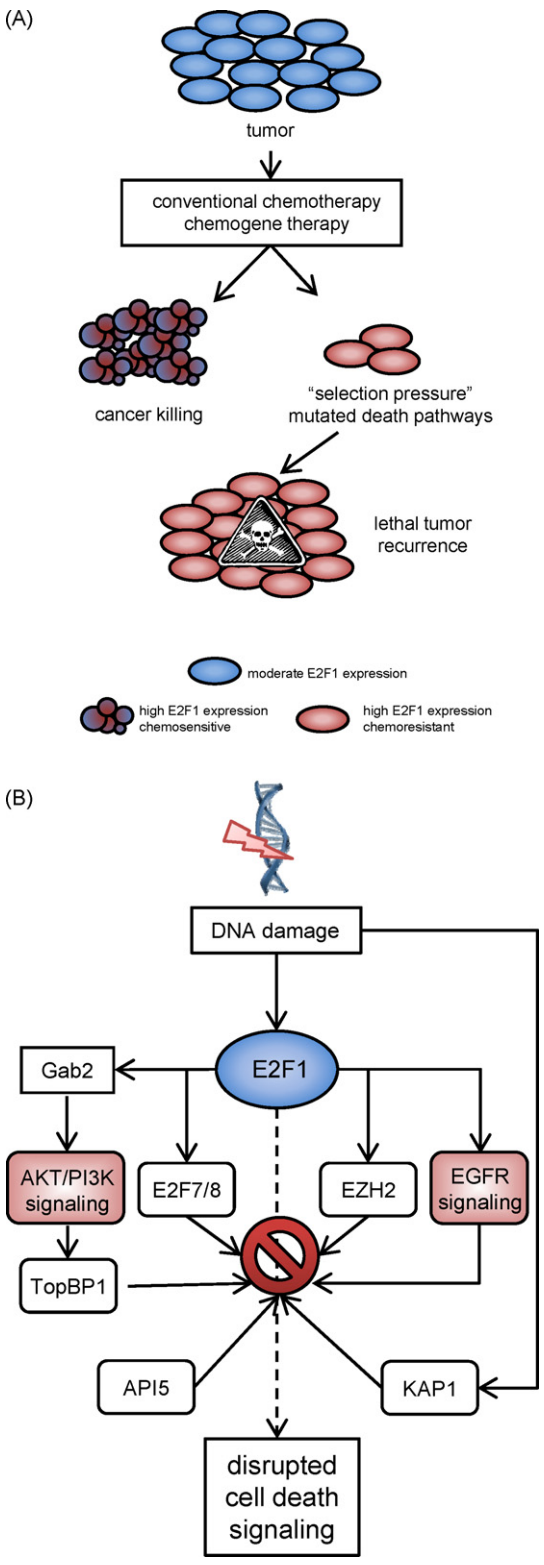

Fig. 5. Resistance to E2F1 driven cell death as a mechanism for tumor evasion. E2F1 triggers cell death and acts as a tumor suppressor, but also promotes survival programs by activating oncogenes. (A) Despite of a potent tumor cell killing effect of conventional chemotherapy in human cancers with deregulated E2F1 expression or *chemogene* (E2F1 + drug) treatment, these therapies have also oncogenic side-effects, particularly in advanced tumors with disrupted cell death pathways, that select for apoptosis-resistant phenotypes, tumor recurrence and progression. (B) Negative feedback loops mediated by E2F1 inducible oncogenic signalling pathways such as AKT/PI3K and EGFR, epigenetic regulators (e.g. EZH2) and anti-apoptotic effector proteins (KAP1, API5) control E2F1-induced apoptosis in response to chemotherapy and provide ways for tumor evasion.

been implicated in restraining E2F1 apoptotic activity (Moon et al., 2006), while EGFR itself was recently shown to be activated by E2F1 through a direct transcriptional mechanism, leading to melanoma metastasis independent from E2F1s proliferative activity (Alla et al., 2010). Here it appears of interest that TopBP1 and AKT are also targets of E2F1, suggesting the existence of a self-control mechanism by which E2F1 modulates its own apoptotic activity in favor of a pRB-independent prosurvival response (Chaussepied and Ginsberg, 2004; Yang et al., 2008).

There is evidence that epigenetic events are also involved in E2F1-triggered anti-apoptotic loops and impede its tumor suppressor function. The E2F1 inducible oncogenic polycomb histone methyltransferase EZH2 antagonizes expression of the E2F1 proapoptotic target Bim, thereby dampening E2F1 apoptosis (Wu et al., 2010). Since EZH2 is induced by doxorubicin treatment in tumor cells (unpublished data), epigenetic silencing of proapoptotic E2F1 target genes may contribute to evasion of cancer cells from drug-induced apoptosis. Knockdown strategies resulting in gene silencing of EZH2 restored E2F1 apoptotic activity (Wu et al., 2010), thus providing a new treatment option for cancer.

Another level of regulation during DNA damage comes from effector proteins that counteract E2F1-induced apoptosis and are not directly E2F1-dependent. KAP1, for example, is a nuclear corepressor interacting with chromatin modifying factors that is phosphorylated by ATM in response to DNA damage. It stimulates formation of the E2F1–HDAC1 complex, inhibition of E2F1 acetylation and apoptosis (Wang et al., 2007a). More importantly, depletion of endogenous KAP1 in pRb-deficient cancer cells increased E2F1 transcriptional activity and sensitized its apoptotic DNA damage response. Moreover, apoptosis inhibitor-5 (Api5) was identified as a suppressor of E2F1-induced apoptosis *in vivo* (Morris et al., 2006). Api5 functions downstream of E2F1 and does not inhibit E2F1 transcriptional activity. This E2F–Api5 functional interaction is conserved from flies to humans, where Api5 expression is often upregulated in tumor cells, in particular metastatic cells, suggesting that it can enable tumor cells to evade E2F1-induced apoptosis.

In this aspect, the identification of physiological regulators of E2F1-induced apoptosis opens potentially important perspectives for future anticancer therapies. Specifically, pharmacological attenuation of signalling routes that block E2F1-induced apoptosis may lead to selective killing of tumor cells that lack functional RB but retain operational apoptotic machinery downstream of E2F1. However, considering the multiple autoregulatory feedback loops initiated by E2F1 itself, it is paradoxically apparent that E2F1 despite all cancer killing activities has the potential to self-activate drug resistance. Indeed, there is recent evidence showing that E2F1 can mediate drug (tamoxifen) resistance in human breast cancer cells (Louie et al., 2010). Consequently, therapies using E2F1 or even conventional anticancer drug treatments which are primarily E2F1-dependent will have oncogenic side-effects, especially on high grade tumors with severe defects in cell death pathways that may, *a priori*, select against apoptotic consequences of deregulated E2F1 activity. Under these conditions E2F1, initially unleashed to fight cancer, can act as a driving force for tumor progression (Alla et al., 2010).

7. Conclusion

Therapeutic success of chemotherapeutic agents depends for a large part on functional translation of DNA damage into efficient cancer cell apoptosis induction. Key molecules like E2F1 have been identified as mediators of these cytotoxic effects in the course of its cancer defense function. In p53-deficient cells, E2F1 is part of a failsafe mechanism at the frontier of apoptosis resistance. Up to recently E2F1 was thought to have a unique tumor suppressor function within the family of E2F transcription factors, but now it is evident that E2F3a and E2F2 have important supporting roles. In this context RB has been characterized to mediate drug-induced apoptosis in an E2F1-dependent manner. Thus it appears that E2F1's ability to induce apoptosis is more deep-seated within the RB-E2F axis than initially assumed. Although E2F1 apoptosis signalling is highly conserved and exceedingly complex, paradoxically the same axis is responsible for an aggressive cancer phenotype, tumor progression and therapy failure. It seems that during cancer progression, E2F1 loses its suppressor function thereby unleashing its oncogenic capabilities that still remain to be determined *in vivo*. Mechanisms that lead to an insensitivity of E2F1-induced apoptosis largely depend on E2F1 itself. This is the major obstacle for *chemogene* therapy and is highly relevant for conventional drug treatment as well. It has to be realized that DNA damage inflicting agents which are commonly used in current chemotherapy regiments mainly trigger the E2F–RB axis – a pathway that is highly carcinogenic. Thus, re-establishment of E2F1s tumor suppressor function by oncogene inhibitors in conjunction with chemotherapy might be a useful option for treatment of apoptosis resistant cancers.

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