*Review*

Cancer Research

# The Dark Side of E2F1: In Transit beyond Apoptosis

David Engelmann and Brigitte M. Pützer

## Abstract

E2F1 plays a critical role in cell-cycle progression and the induction of apoptosis in response to DNA damage. The latest evidence has uncovered that this tumor suppressor is most relevant for cancer progression and chemoresistance. Increased abundance of E2F1 triggers invasion and metastasis by activating growth receptor signaling pathways, which in turn promote an antiapoptotic tumor environment. The data shed light on the molecular mechanisms underlying E2F1-induced prometastatic activity and predict its radical switch from a mediator of cell death toward an accelerator of tumor progression. This raises the perspective of new drug targets at late-stage cancer. *Cancer Res; 72(3); 571–5. ©2012 AACR.*

## Expanding the E2F Paradigm

More than 2 decades of experimentation link E2F activity to retinoblastoma tumor suppressor (RB)–dependent cell-cycle control. E2F proteins are situated downstream of growth factor signaling cascades, where they regulate genes required for cell-cycle progression by acting either as transcriptional activators or as repressors. Activating members of the E2F family (E2F1-3) can contribute to oncogenic transformation of rodent embryonic fibroblasts and tumorigenesis when overexpressed. The absence of activating E2Fs in flies or mammalian fibroblasts causes cell-cycle arrest, but this block is alleviated by removing repressive E2F or the tumor suppressor p53. The idea that E2F function is indispensible for cell proliferation is under debate and has dominated discussions for years.

Recent work from Chen and colleagues in knockout mouse models is challenging the current E2F paradigm. The researchers showed that in retinal progenitor cells deficient for all activating *E2Fs*, proliferation is bypassed by MYCN (1). Instead, activating E2Fs have an unexpected prosurvival role during retinal development via induction of the apoptosis inhibitory Sirt1-p53 axis. Similar results were obtained from transgenic mice with conditional *E2F* triple deficiency in embryonic stem cells. Thus, E2F1-3 function is, contrary to the current view, expendable for cell division in the majority of cell types present during organ development and embryogenesis, but it is necessary for suppressing cell death (2). Although these studies were accomplished in normal tissues, they might teach us a lesson about the role of E2Fs in cancer. On the basis of recently published research, we should challenge the prevailing view that cell proliferation is the major and most important oncogenic mechanism of E2Fs. Even if this function may have evolved during tumor development, we are only beginning to understand how E2F family proteins participate in other, as yet uncharted, biologic processes that contribute to tumor promotion and, more importantly, to tumor progression.

## Switching Duties: A Tumor Suppressor Contributes to Cancer Progression

In the past, E2F1 in particular was recognized as a strong regulator of apoptosis after DNA damage in all types of human cancer (3). Most human tumors harbor functionally inactivated RB, resulting in deregulated E2F1 in the transformed cells. In this context, E2F1-induced apoptosis signaling is viewed as a fail-safe mechanism that opposes proliferation and oncogenesis through its capacity to activate the p53/p73 pathway of intrinsic cell death. Whether the balance of E2F1 activity in a specific tissue inhibits or promotes tumorigenesis is most likely dependent upon the context of pro- versus antiapoptotic signals received by cells at a given time. It has been shown that tumor cells, especially from advanced lesions, exhibit severe defects in the cell death pathways that are normally activated by E2F1, which may otherwise select against apoptotic consequences of deregulated E2F1 in the absence of RB (4). The first evidence indicating that RB and oncogenic signals cooperate to suppress E2F1-induced apoptosis came from data on *Drosophila* mutant flies (5). Researchers have shown that E2F1 tumor suppressor activity can be prevented by EGFR/Ras/Raf signaling during normal development. Another critical inhibitor of proapoptotic E2F1 target genes in serum-stimulated fibroblasts and human tumor cells *in vitro* is the phosphoinositide 3-kinase (PI3K)/AKT pathway, also activated by EGFR, whereas E2F1-regulated genes involved in the proliferative program are not affected (6). AKT is also a target of E2F1 (7), suggesting the existence of a self-control mechanism by which E2F1 modulates its own apoptotic activity in favor of an RB-independent prosurvival response.

**Authors' Affiliation:** Department of Vectorology and Experimental Gene Therapy, Biomedical Research Center, University of Rostock, Rostock, Germany

**Corresponding Author:** Brigitte M. Pützer, Department of Vectorology and Experimental Gene Therapy, Biomedical Research Center, University of Rostock, Schillingallee 69, D-18057 Rostock, Germany. Phone: 49-381-4945066; Fax: 49-381-4945062; E-mail: brigitte.puetzer@med.uni-rostock.de

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Abnormalities in *E2F1* gene expression and/or *E2F1* gene amplification are seen in many types of human cancer. Respective studies indicate that increased E2F1 expression is frequently and predominantly associated with high-grade tumors or metastases and unfavorable patient survival prognosis. Lee and colleagues showed that high expression of E2F1 and its associated target genes predict the change from superficial to invasive progression of bladder tumors (8). Together with the finding that E2F binding sites are enriched in promoters of genes whose expression is strongly associated with invasive tumors, the research of Lee and colleagues suggests that E2F1 is functionally connected to cancer invasiveness. Further hints that derepression of E2F1 signaling can pave the way for a progressive cancer disease come from recent studies showing that loss of the RB/E2F-negative regulator p16 in patients with gastrointestinal stromal tumors of the stomach has a major impact on tumor-related survival (9). These correlations can be linked to the evasion of advanced tumor cells from E2F1-related apoptosis. Dysregulated E2F1 has been shown in combination with p53 deficiency or activated Ras to accelerate tumorigenesis. Furthermore, reduced expression of genes belonging to the E2F1 apoptotic program in breast and ovarian cancer patients coincides with poorer survival outcomes (6). Together, these findings emphasize the importance of E2F1 signaling as a mechanism for establishing cancer drug resistance in association with tumor progression and encourage the view that E2F1 has an additional oncogenic property independent of its ability to simply stimulate aberrant growth.

Our recent *in vivo* data provide the first functional evidence that E2F1 is crucial in transformed cells for local invasion and to form distant metastases (10). This study showed that knockdown of endogenous E2F1 in a metastatic melanoma model severely reduces the migratory and invasive potential of skin cancer cells, whereas cell proliferation is not affected. Melanocytic tumors in mice with blocked E2F1 expression showed a drastic reduction of pulmonary metastases, indicating that repression of E2F1 is sufficient to attenuate the ability of melanoma cells to metastasize. Now the question arises of how E2F1 accomplishes tumor progression and whether this represents a more general paradigm beyond cell-cycle regulation. In malignant melanoma, E2F1-dependent progression was found to be mediated through the upregulation of EGFR and activation of the cytoplasmic Ras/mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) and PI3K/AKT signaling cascades, as well as *B-Myb* transactivation *in vitro* (10). It seems that the EGFR ligand HBEGF is also a transcriptional target of E2F1, providing the possibility for autocrine stimulation of EGFR signaling in aggressive skin cancer. In support of this finding, Sharma and colleagues uncovered the androgen receptor, which is essential for the initiation and progression of prostate cancer, as a gene under control of E2F1 in both cultured tumor cells and xenografts (11). E2F1 was found to bind the androgen receptor promoter, and this occupancy was enriched in the absence of RB. In-depth analysis of human castrate-resistant prostate cancer metastases revealed that loss of *RB* during cancer progression correlates with increased E2F1 and androgen receptor levels. These data convincingly show that EGFR and androgen receptor signaling is essential for malignant cells overexpressing E2F1 to leave their primary location and open up the possibility that other cellular or nuclear growth factor receptors might be regulated by E2F1 in other types of cancer (Fig. 1). Meanwhile, a cell cycle–independent but developmentally relevant role for the RB/E2F pathway was found for neural precursor migration in embryonic mouse models (12). Thus, regulation of cell motility and invasiveness by the RB/E2F pathway in cancer may reflect a physiologic function, which is tightly controlled during organ development and apparently becomes reactivated during tumor progression.

## E2F1-Binding Cofactors and Cancer Progression

Recently, several studies have increased our understanding of the mechanistic control of E2F-driven gene expression by cofactors that bind E2F1, thereby having a profound influence on its ability to mediate tumor progression. One category of nuclear cofactors acts by enhancing E2F1's ability to transactivate target gene promoters. Revenko and colleagues identified the AAA nuclear coregulator cancer-associated protein (ANCCA) as a coactivator of E2F1 in human cancer cells. It directly interacts with E2F1 and is important for target gene expression *in vitro* (13). ANCCA preferentially associates with acetylated histones and serves as a "pioneer" factor for E2F1 by anchoring at specific chromatin locations. A clinical study from the same group correlates high levels of ANCCA with triple-negative breast cancers, tumor metastasis, disease recurrence, and poor survival (14). The study revealed that E2F1-regulated oncogenes, such as the polycomb protein EZH2 and B-Myb, are highly expressed in these tumors, whereas knockdown of ANCCA leads to their downregulation. Another cofactor of E2F1 gene expression that is strongly implicated in tumorigenesis and cancer progression is the nuclear receptor coactivator 3 (NCOA3), alias ACTR (15). Because both cofactor-encoding genes are also transactivated by E2F1 itself (16, 17), this opens up the feasibility of feedback loops constituting a deadly alliance, rendering tumors more aggressive and untreatable. Because nuclear EGFR interacts with E2F1, leading to the activation of *B-Myb* expression, it may also be considered as a cofactor, which, as a target of E2F1, facilitates tumor progression partly via triggering E2F1-dependent gene expression. Other data suggest that cofactor-driven E2F1 transcriptional activity of genes facilitating tumor progression can be induced via nicotinic acetylcholine receptor (nAChR) signaling (18). Arrestin beta 1 (ARRB1) was shown to be translocated into the nucleus and to be directly associated with E2F1 upon nAChR stimulation by nicotine in lung cancer cells. ARRB1-E2F1 complexes were significantly more abundant in non–small cell lung carcinoma tissue from smokers relative to matched normal lung tissue. The ARRB1 protein has been shown to promote multiple steps of cancer progression, including

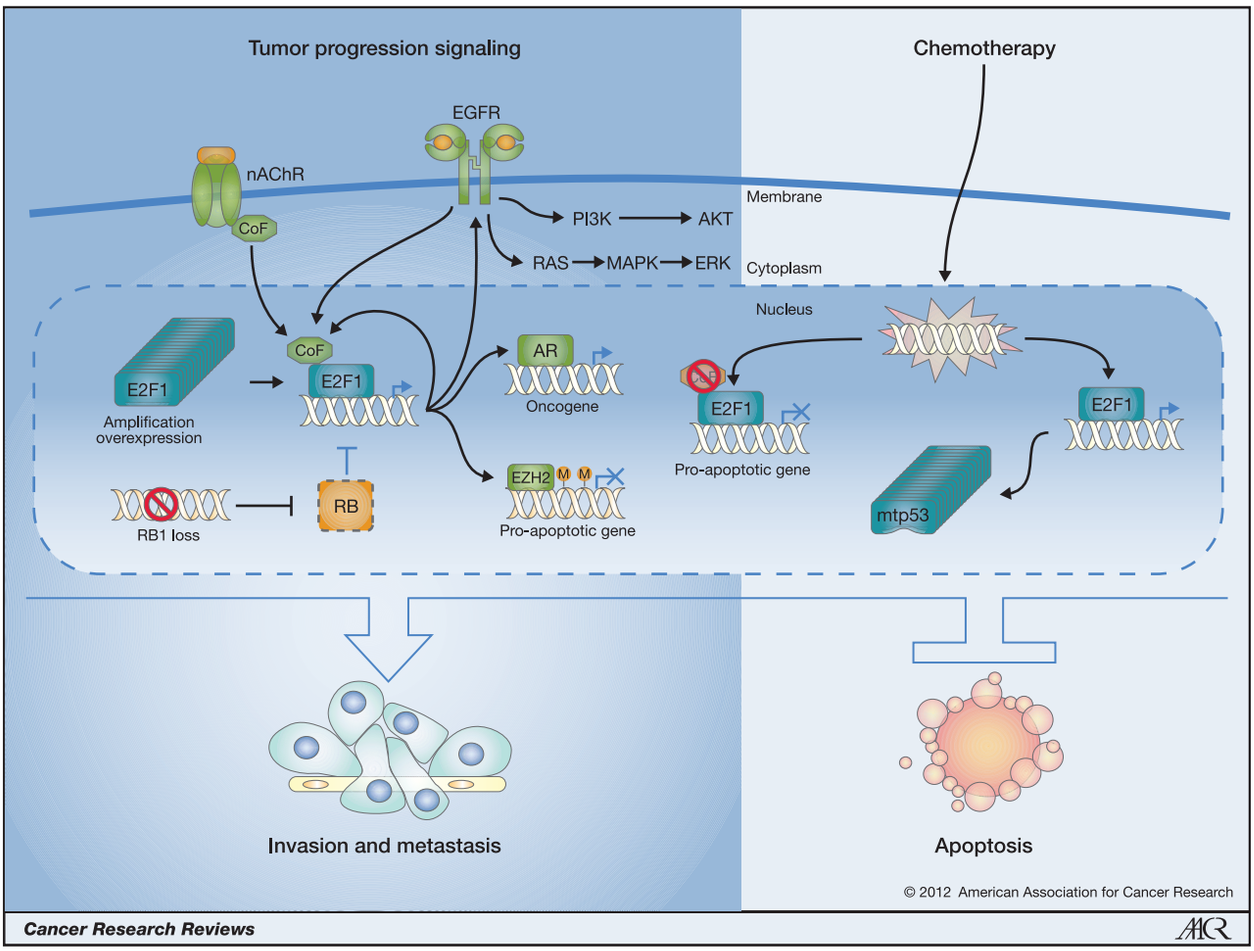


Figure 1. Transition of E2F1 from a tumor suppressor to an inducer of cancer progression. In tumor cells that have lost the *RB* locus and simultaneously overexpress E2F1, oncogenic E2F1 target genes, such as growth receptors (EGFR, androgen receptor), polycomb group proteins (EZH2), and nuclear cofactors (CoF), are highly upregulated. This upregulation leads to the activation of cytoplasmic (PI3K/AKT and RAS/MAPK/ERK) and nuclear signaling cascades related to invasion and metastasis. By activating cofactors, E2F1 constitutes feedback loops, which promote its prometastatic function. The same pathways abrogate its apoptotic function. Under these conditions, chemotherapy enforces expression of oncogenes, such as mutant p53 (mtp53), and thereby favors resistance toward apoptosis. The antiapoptotic response is supported by the loss of E2F1 cofactors required for proper activation of death genes.

invasion and metastasis across a range of cancers (19) and is, consistent with its oncogenic activity in cooperation with E2F1, expressed at the highest levels in several metastatic cancers. Besides cofactors that function as positive regulators of E2F1, receptor-interacting protein of 140 kDa (RIP140) seems to be a first example that such proteins are also critical for repression of metastatic behavior (20). RIP140 binds to the RB interaction domain of the E2F1 protein, thereby mimicking RB function. Corresponding with the activity of E2F1 in aggressive cancers, low levels of RIP140 are associated with high levels of E2F1 target genes in basal-like breast tumors that frequently metastasize and exhibit high mortality rates, whereas RIP140 expression is high in the luminal subtype.

From this body of knowledge, E2F1 and its cofactors, independent of whether they are activators or repressors, have entered the spotlight as drug targets. Selective interference with E2F1 or ANCCA has been shown to prevent metastasis (10) or tumor growth (14, 21). Therefore, it is reasonable to search for small-molecule inhibitors that disrupt the binding of E2F1 to promoters or the interactions between E2F1 and cofactors.

## Metastasis Suppressor MicroRNAs Target E2F1

To date, it is not clear how E2F1 itself is upregulated in metastatic cancer cells. Here, the latest evidence points toward a contribution of microRNAs. Mir-205 was identified as the microRNA with the highest degree of downregulation in melanoma metastases, and it prevents tumor progression via direct inhibition of E2F1 expression (22). The small noncoding microRNA let-7g is known to counteract cancer cell migration and is clearly downregulated in metastases from hepatocellular carcinoma patients (23). This candidate metastasis suppressor was shown to inhibit E2F1-dependent

cell migration via targeting the E2F1 activator HMGA2 (24). These studies clearly emphasize that E2F1 is at the frontier of tumor progression targeted by microRNAs opposing metastasis.

## E2F1 Mediates Chemoresistance

As cancers of advanced stage are commonly resistant against DNA-damaging agents, E2F1 links metastasis to chemoresistance. Indeed, indications are clear that drug-resistant tumor cells display an upregulation of the oncogenic E2F1-signaling network, which mediates therapy evasion (25). Microcephalin 1 (MCPH1) cooperates with E2F1 to induce genes involved in DNA repair and apoptosis upon chemotherapy in normal and tumor cells through complex formation on the promoters of these genes (26). Considering the potential impact of cofactors on E2F1's transcriptional activity, it is intriguing that decreased levels of MCPH1 are associated with genomic instability and metastasis (26). In addition, EZH2, shown to antagonize expression of the E2F1 proapoptotic target *BIM* and to drive metastasis (27), abrogates the DNA damage response (28). Because EZH2 is induced by E2F1 as well as genotoxic stress, it is reasonable, if not logical, to assume that anticancer drugs can even potentiate apoptosis evasion and cancer metastasis. This assumption also becomes apparent from a recent article by Bug and Dobbelstein (29). The tumor suppressor p53 is mutated in about half of all human malignancies and accumulates to levels that by far exceed those of wild-type p53. The consequences of mutant p53 accumulation include increased chemoresistance and enhanced ability to metastasize (30). In this context, it seems interesting that E2F1 binds the *TP53* promoter and is necessary for efficient induction of mutant p53 upon drug treatment *in vitro* (29). The findings are in line with a cell context-dependent synergism between E2F1 and DNA-damaging agents in the upregulation of genes that are related to cell survival and tumor progression (3, 31).

## Future Perspectives

Together, the research implies that current therapies based on the enforcement of apoptosis may not be suitable for the eradication of aggressive cancer cells. In a cellular context in which E2F1's prometastatic signaling becomes predominant, conventional anticancer drug therapy will possibly render adverse effects by supporting cancer progression (Fig. 1). Consequently, full knowledge of the molecular mechanisms underlying E2F1-induced tumor progression in an individual cancer context is vital to avoid therapy failure and to improve antimetastatic treatment.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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