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Review

Transcription addiction: can we garner the *Yin and Yang* functions of E2F1 for cancer therapy?

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Classically, as a transcription factor family, the E2Fs are known to regulate the expression of various genes whose products are involved in a multitude of biological functions, many of which are deregulated in diseases including cancers. E2F is deregulated and hyperactive in most human cancers with context dependent, dichotomous and contradictory roles in almost all cancers. Cancer cells have an insatiable demand for transcription to ensure that gene products are available to sustain various biological processes that support their rapid growth and survival. In this context, cutting-off hyperactivity of transcription factors that support transcription dependence could be a valuable therapeutic strategy. However, one of the greatest challenges of targeting a transcription factor is the global effects on non-cancerous cells given that they control cellular functions in general. Recently, there is growing realization regarding the possibility to target the oncogenic activation of transcription factors to modulate transcription addiction without affecting the normal activity required for cell functions. In this review, we used E2F1 as a prototype transcription factor to address transcription factor activity in cancer cell functions. We focused on melanoma considering that E2F1 executes critical functions in response to UV, an etiological factor of cutaneous melanoma and lies immediately downstream of the CDKN2A/pRb axis, which is frequently deregulated in melanoma. Further, activation of E2F1 in melanomas can also occur independent of loss of CDKN2A. Given its activated status and the ability to transcriptionally control a plethora of genes involved in regulating melanoma development and progression, we review the current literature on its differential role in controlling signaling pathways involved in melanoma as well as therapeutic resistance, and discuss the practical value of weaning melanoma cells from E2F1-mediated transcription dependence for melanoma management.
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Facts

- Cancer cells are addicted to transcription to maintain enhanced survival needs
- E2F1 transcriptionally regulates many biological functions deregulated in cancers
- E2F1's role in survival and death is context dependent
- Deregulation of CDKN2A/pRb axis highlights the importance of E2F1 in melanoma

Open Questions

- Are the biological functions of a transcription factor different in normal versus cancer cells?
- Is it possible to realistically tease out oncogenic function from the normal function of transcription factors?
- Is it possible to therapeutically target transcription factors?

E2F1's Early History and Role in Cancer

In 1986, E2F was identified as a cellular transcription activator binding to adenovirus E2 promoter.[1] Since then, eight mammalian family members have been identified. On the basis of their ability to regulate downstream target genes, they are classified into two groups, activators (E2F1-3) or repressors (E2F4-8; see Figure 1).[2] As the archetype member, E2F1 is the most thoroughly investigated. The ability to promote cell cycle progression through timely regulation of genes required for DNA synthesis at the G1/S boundary, and contribute to apoptosis induction by cooperating with p53 or p73 makes E2F1 a special member of this family.[3] E2F1 has typical domains for its transcription factor activity including, DNA-binding domain (DBD) next to the N terminus, and transactivation domain (TAD) located in the C terminus (shown in Figure 1). Between these two domains is the homo–hetero dimerization domain, which is important for its dimerization with DNA-binding protein, DP1. In addition,

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Abbreviations: BRCT, BRCA1 carboxyl-terminal; DREAM, downstream regulatory element antagonist modulator; Gab2, Grb2-associated binder 2; HAT, histone acetyltransferase; MMP, matrix metalloproteinase; MTX, methotrexate; NSCLC, non-small-cell lung carcinoma; PRMT5, protein arginine methyltransferase 5; TMECG, tyrosinase-processed anti-folate prodrug 3-O-(3,4,5-trimethoxybenzoyl)-(-)-epicatechin; TopBP1, Topoisomerase II β-binding protein; VHL, Von Hippel–Lindau

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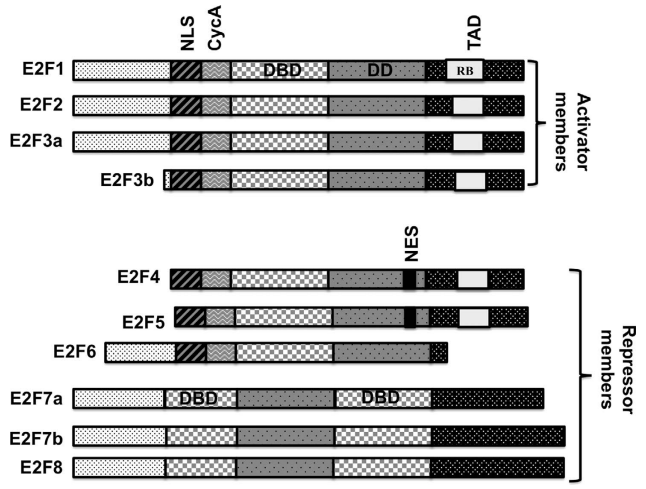

Figure 1 Functional domains of E2F transcription factor family. On the basis of their ability to regulate downstream target genes, E2F family members are classified into two groups, activators (E2F1-3a) or repressors (E2F3b-8). As transcription factors, they all have the DBD, NLS, DD (dimerization domain), TAD, NES (nuclear exclusion signal; modified from Chen *et al.*[2])

there are distinct domains responsible for its regulation and degradation such as the cyclin A binding site near the nuclear localization signal and the pRb binding domain juxtaposed with the $p14^{ARF}$ binding region, both of which are in the TAD. Binding of the former prevents binding of the latter and consequential degradation.[4] E2F1 executes most of its biological functions through its transcription activator ability. E2F1 is known to upregulate several genes involved in cell cycle, DNA synthesis and replication, checkpoint control, DNA damage and repair, apoptosis, autophagy, self-renewal, development and differentiation, and so on.[3,5–9] (shown in Figure 2). However, E2F1 also has transcription-independent activities that facilitate DNA repair or induce autophagy and apoptosis.[10–12] E2F1 knockout animals develop normally, display testicular atrophy, exocrine gland dysplasia, and exhibit maturation stage defect in thymocyte apoptosis suggesting a role for E2F1 in apoptosis.[13,14] The role of E2F1 in recruiting other transcription factors and co-factors has not been thoroughly investigated and certainly deserves more attention, which is more than likely to increase the biological complexity of E2F1.

E2F1 have been found to be deregulated in many types of cancers (see Table 1), including hepatocellular carcinoma, non-small-cell lung carcinoma (NSCLC), cervical cancer, glioblastoma, pancreatic cancer, renal, breast, and ovarian cancer.[2] E2F1 has contradictory roles in cancer. For example, E2F1 knockout mice develop reproductive tract sarcomas, lung adenocarcinomas, and lymphomas.[13] Contrastingly, the upregulation of E2F1 has been associated with inactivation of the tumor suppressor Von Hippel–Lindau (VHL) gene, and key mutations underlying renal cancer.[15] Although VHL may regulate E2F1 in HIF-dependent and -independent ways,[15] E2F1 overexpression in high-grade clear cell renal cell carcinoma tissues is known to contribute to activation of matrix metalloproteinase (MMP) 2 and MMP9, which suggests a role in tumor progression.[16] Although high expression of E2F1 in VHL-defective renal cancer is associated with senescence and has been suggested to be protective in the context of renal cancer, caution is warranted in designating E2F1 as oncogene or tumor suppressor gene for which clearly more extensive investigation is required.[17] In lung cancer, highly significant association between E2F1 and the ATP-binding cassette sub-family G member 2 has been found implying that E2F1 could temper response to chemotherapeutic drugs.[18] Interestingly, E2F1 overexpression is frequently found in NSCLC and E2F1-inducible miR-449 has a tumor suppressive role[19] although, there is no correlation between E2F1 protein expression and clinical outcome including progression-free survival.[20] Considering the double-edged role of E2F1 in regulating cellular growth and death homeostasis, the actual effects that E2F1 may have on human malignancies can be difficult to predict.

Post-Translational Modifications of E2F1 Controls its Activities

Phosphorylation. Cells regulate protein functions and transmit signals through transient control of phosphorylation/dephosphorylation. Published studies show that regulation of E2F1 availability and activity are highly dependent on multiple post-translational modifications. As shown in Figure 3, progression through the cell cycle is dependent on E2F1 status and fate, which are intimately tied to cell cycle progression. The motifs and their corresponding modifications control E2F1's fate perfectly in normal cells. Thus, after pRb is hyperphosphorylated, phosphorylation of E2F1 at Serine-332 and -337 by cyclin D/cdk4/6 complex at the G1/S transition point increases E2F1 stability and prevents pRb binding. Sequential acetylation of E2F1 at Lysine-117, 120, and 125 further stabilizes E2F1 and increases DNA-binding ability of E2F1/DP heterodimer. In late S phase, cdk2 recruited by cyclin A phosphorylates E2F1 at Serine-375, which causes the release of DP protein and reduces DNA-binding ability of E2F1 itself. This process facilitates the binding of $p14^{ARF}$ to the carboxyl terminus of E2F1 and promotes subsequent binding of the ubiquitin protein ligase $p45^{skp2}$ to the amino terminus, which leads to the degradation of E2F1 in S–G2 phase.[4] The importance of these post-translational modification sites is also reflected in regulating E2F1's fate under stress. For example, in response to DNA damage, ATM/ATR kinases phosphorylate E2F1 at Serine-31, which allows E2F1 accumulation probably by inhibiting binding and or degradation through $p45^{skp2}$. During this process, 14-3-3τ, a phospho-serine-binding protein, may have an important role. 14-3-3τ has been found to interact with ATM-phosphorylated E2F1 during DNA damage to inhibit its ubiquitination.[21] Besides, it regulates the expression of several E2F1 apoptotic targets, including p73, Apaf-1, and caspases.[22] E2F1 is also phosphorylated by CHK2 at serine-364, which causes protein stabilization and transcriptional activation.[23] Considering that ATM can be transcriptionally regulated by E2F1 leading to CHK2 phosphorylation, the ATM-CHK2-E2F1 axis may form a positive feedback loop in response to genotoxic stress and regulate apoptosis in damaged cells.[24,25] E2F1 functions in a positive feedback loop by binding to the promoter of the phosphatase inhibitor, CIP2A

that inhibits E2F1's phosphorylation on serine-364 to inhibit p53-mediated senescence.[26]

Other modifications. Acetylation-mediated regulation of E2F1 is complicated as E2F1 has been reported to interact with multiple histone acetyltransferases (HATs), including PCAF, CBP/p300, Tip60, and GCN5 to execute different roles.[27–30] SIRT1/PCAF interaction controls the E2F1/p73 apoptotic pathway in response to DNA damage.[31] Depletion of CBP and p300 inhibits binding of E2F1 to BRCA1 and RAD51 promoters, key genes of homologous recombination. Association of E2F1 with Tip60 or GCN5 is involved in nucleotide excision repair.[32,33] Although in most cases, acetylation of E2F1 by HATs is related to apoptosis or DNA repair, it is also involved in the angiogenic process through VEGF stimulation,[34] which further increases the complexity of the roles of E2F1. Besides phosphorylation and acetylation, studies show that E2F1 is epigenetically regulated through methylation. Lysine-185 was the first methylation site found on E2F1, which is methylated by SET9 and demethylated by LSD1.[35] Methylation destabilizes E2F1 during DNA damage and prevents activation of its proapoptotic target p73, whereas demethylation has the opposite role.[35] Work by Cho *et al.*[36] showed that methylation of arginine-111 and 113 by protein arginine methyltransferase 5 (PRMT5) reduces E2F1 stability. In PRMT5-depleted cells, there is enhanced E2F1 and apoptosis, and decreased growth.[36] Another antagonistic methylation site, arginine-109, methylated by PRMT1, contributes to increased protein half-life and E2F1-dependent apoptosis.[37]

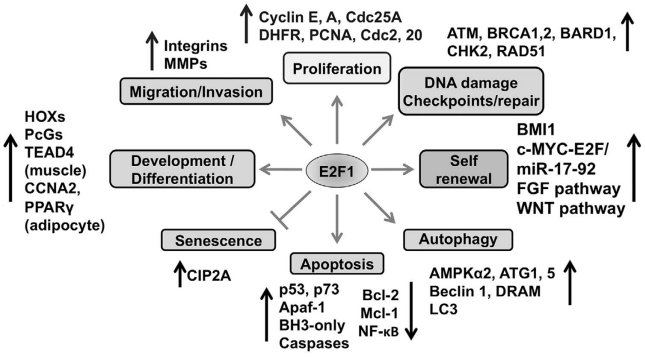

Figure 2 E2F1 regulates several biological functions. E2F1 is known to upregulate many genes involved in cell cycle, DNA synthesis and replication, checkpoint control, DNA damage and repair, apoptosis, autophagy, self-renewal, development and differentiation, and so on.[3,5–9] E2F1 also represses antiapoptotic genes or survival pathways to induce apoptosis. Downregulation of E2F1 is related to senescence considering its role in promoting cell cycle progression

Table 1 Differential expression and levels of E2F1 in various cancers are associated with different functions and outcomes

Human cancer	E2F1 expression	Biological functions
Bladder cancer	Controversial in publications	Low E2F1 reactivity in tumor caused increased risk of progression to metastasis;[84] expression correlated with proliferation in superficial TCCs[85]
Breast cancer	Increase[85–87]	Poor survival and prognostic indicator;[85–87] overexpression induced apoptosis in human breast cancer cells[88]
Cervical cancer	Increase; gene amplification[89–91]	Aberrant cell cycle regulation;[92] mediates overexpression of emerging markers for detection of high-grade cervical disease[93] mediates miRNAs in response to HPV E7[94]
Colon cancer	Decrease[2,11] overexpression in lung and liver metastases of human colon cancer[95]	Inversely associated with tumor growth;[85,96] upregulates c-Myc and $p14^{ARF}$ and induces apoptosis in colon cancer cells[97]
Esophageal cancer	Increase; gene amplification[98]	Poor survival;[99,100] positively associated with cell proliferation but not apoptosis;[101] however, positively correlates with apoptosis and inversely correlates with cell proliferation in adenocarcinomas of Barrett esophagus[102]
Gastric adenocarcinoma	Increase[103–105] Higher expression in early stage I–II and lower expression in later stages[106]	Adenovirus-mediated E2F1 overexpression induces apoptosis;[107] overexpression suppresses tumor cell proliferation[108,109]
GI stromal cancer	Increase[110]	Increased cell proliferation and adverse prognosis[110,111]
Glioblastoma	Increase[112]	Overexpression increases chemosensitivity[113]
Hepatocellular carcinoma (HCC)	Increase; gene amplification[114–116]	Correlated with enhanced tumor cell apoptosis;[114] tumor-promoting roles in HCC cell lines or mouse models[117–121]
Lung cancerNSCLC	Increase;[122,123] gene amplification[124]	Growth-promoting factor associated with poor prognosis;[125] overexpression induces apoptosis[126]
SCLC	Increase[100,127]	Involved in tumorigenesis by activation of EZH2 oncogene EZH2[128–130]
Melanoma	Increase; gene amplification[42,47]	Overexpression induces apoptosis and growth inhibition;[73,131–133] induces autophagy;[11] controls proliferation by regulating AKT phosphorylation;[66] associated with progression and metastasis[50]
Oral SCC	Increase[134]	Associated with increased overall survival[135]
Ovarian cancer	Increase[136–138]	Overexpression induces apoptosis in human ovarian cancer cells;[88] mediates cell cycle deregulation in high-grade serous ovarian carcinomas;[136] determines balance between proliferation and cell death[139]
Pancreatic ductal carcinoma	Increase[140]	Tumor promoting and poor survival;[59] overexpression induces apoptosis and increases chemosensitivity in pancreatic cancer cells[60,61]

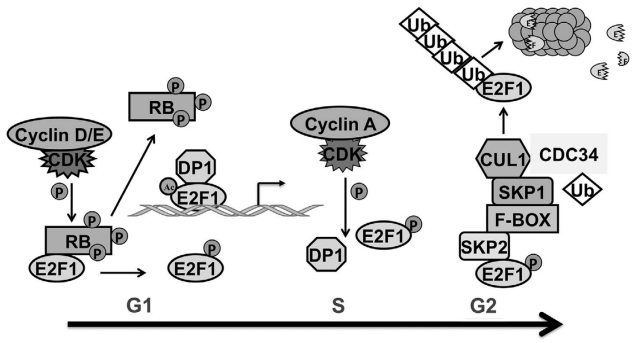

Figure 3 Involvement of E2F1 in cell cycle progression. After pRb is hyperphosphorylated, phosphorylation of E2F1 at Serine-332 and -337 by cyclin D/cdk4/6 complex at the G1/S transition point increases the stability of E2F1 and prevents pRb binding. Sequential acetylation of E2F1 at Lysine-117, -120, and -125 sites further stabilizes E2F1 and increases DNA-binding ability of E2F1/DP heterodimer. In late S phase, cdk2 recruited by cyclin A phosphorylates E2F1 at Serine-375, which causes the release of DP protein and reduces DNA-binding ability of E2F1 itself. This process facilitates the binding of $p14^{ARF}$ to the carboxyl terminus of E2F1 and promotes subsequent binding of $p45^{skp2}$ to the amino terminus and leads to the degradation of E2F1 in S–G2 phase[4]

CrossTalk between post-translational modifications. Multiple post-translational modification sites have been observed, yet there are still many unanswered questions regarding the precise roles of these modifications in controlling the contradicting functions of E2F1. First, is there crosstalk between these sites? Phosphorylation by ATM/ATR and Chk1/Chk2 kinases, together with acetylation, has a positive role in E2F1 stability and activity under DNA damage conditions. Methylation at lysine-185 inhibits acetylation and phosphorylation at distant sites and stimulates ubiquitination and subsequent degradation.[35] Interestingly, pre-acetylated or pre-phosphorylated E2F1 is poorly methylated, suggesting that the cooperation between phosphorylation and acetylation may exclude lysine methylation. Second, how does crosstalk between the modification sites control E2F1's biological role? The competition between PRMT1 and PRMT5 for E2F1 methylation may provide some answers. Although PRMT1 methylation increases E2F1 half-life and augments the expression of E2F1-dependent proapoptotic genes, PRMT5 methylation inhibits expression and favors proliferation. In cells with DNA damage, increased PRMT1 binding and methylation hinders the binding and methylation by PRMT5. Accordingly, apoptosis is induced. Cyclin A binding prevents PRMT1 binding but not PRMT5. The latter methylates E2F1 at arginine-111 and 113, which further affects the accessibility of PRMT1. Therefore, cells are directed to enter the proliferative cycle. This methylation mark is recognized by the Tudor domain protein p100-TSN, which further suppresses apoptosis.[37] Collectively, these lines of evidence partly explain the relevance of post-translational modification and switch of E2F1's activity towards regulating cell survival or apoptosis. However, further studies including *in vivo* modeling will be required to test whether these cell culture observations can be validated. Moreover, whether these post-translational modification sites have any clinical relevance is an intriguing question. Although several studies have examined the clinical relevance of E2F1 through assessment of message and protein levels of human tumor samples, there is a dearth of information regarding the post-translational modification status in clinical samples. Given the importance of post-translational modification in regulating E2F1's activity and biological effects, it is important that future studies examine the clinical relevance of various post-translational modifications of E2F1.

E2F1 in Melanoma

Malignant melanoma is infamous for its aggressiveness, high metastatic potential, and resistance to standard cancer therapies like radiation or chemotherapy.[38] Although historically speaking, E2F1 has been associated with oncogenic function in melanoma, more recent evidence suggests that it is more complex in that it can differentially promote or inhibit biological functions associated with primary or metastatic phenotype. Loss of E2F1 and E2F2 expression is a tumor suppressive mechanism in melanocytes as it leads to withdrawal from cell cycle and terminal differentiation. This occurs more effectively in cells with eumelanin than cells with pheomelanin.[39] Increased CDK inhibitor activity and the resulting loss of E2F1 function is a characteristic melanocyte senescence program that is induced by cAMP pathway.[40,41] High level of C-MYC and the associated low level of the phosphatase PP2A protein in human melanomas is believed to suppress oncogene-induced senescence.[42] These observations implicate high levels of E2F1 in oncogene-induced senescence. However as a direct transcriptional target of C-MYC, E2F1 has a negative regulatory role in hTERT regulation and senescence induction.[43–45] The Halaban group reported that high E2F1 level in melanoma cells was associated with a fivefold higher DNA-binding activity compared with melanocytes in culture.[46] Increased expression of E2F1 in melanoma has been attributed to increased gene copy number of E2F1 in malignant melanoma.[47] Given that oncogenic addiction of melanoma cells to MDM2 is dependent on E2F1, it has been suggested to serve as a biomarker to stratify patients who may receive p53-MDM2 inhibitors for treatment.[48] E2F1 regulates melanoma cell survival genes such as ASK/Dbf4.[49] E2F1 also contributes to melanoma metastasis through the induction of epidermal growth factor receptor.[50] Although, E2F1 is a transcription factor and its exact role in melanoma is not fully understood yet, there is evidence of complicated crosstalk between E2F1 and several deregulated pathways in melanoma. These findings are suggestive of the important role E2F1 may have in regulating disease progression and drug resistance as it relates to malignant melanoma. From the known interactions between E2F1 and various signaling pathways (Figure 4), it is not difficult to see that the ability of E2F1 to control a multitude of biological processes makes its role in cancer cells rather complex.

Interaction with Ras–Raf–MEK–ERK signaling. The high prevalence of BRAF and NRAS mutations indicates the importance of Ras–Raf–MEK–ERK pathway in melanoma. ERK is believed to lie upstream of pRb-E2F1 as ERK1/2 is known to upregulate the expression of cyclin D1,[51] which induces the activation of CDK4/6 and subsequently

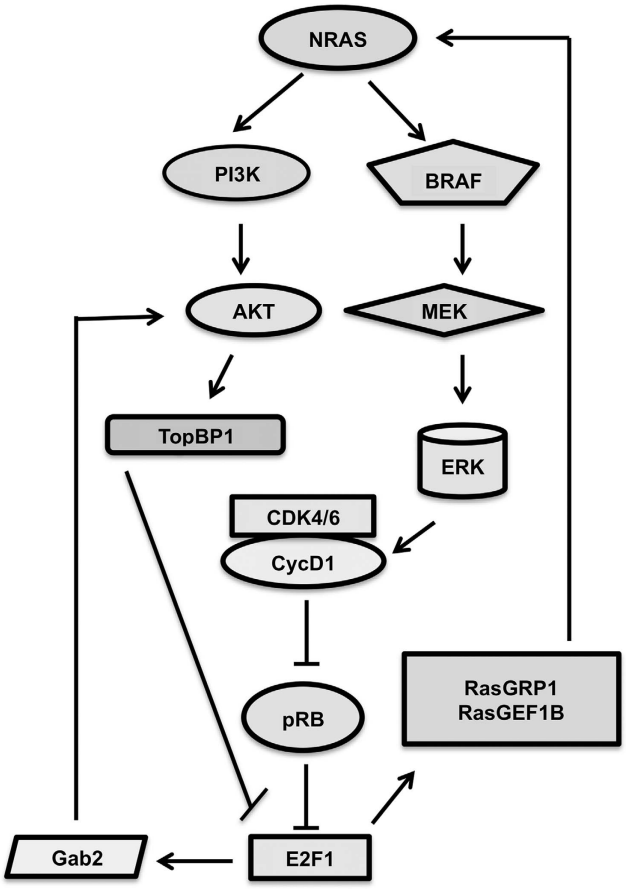

Figure 4 E2F1 is involved in crosstalk with Ras–Raf–MEK–ERK and PI3K–AKT pathways. E2F1 promotes sustained AKT activation through Gab2, whereas AKT in turn inhibits E2F1-mediated apoptosis by activation of TopBP1. ERK1/2 is known to upregulate the expression of cyclin D1,[51] which induces the activation of CDK4/6 and subsequently phosphorylates pRB to release E2F1. Meanwhile, E2F1 also regulates ERK activation via transactivation of RasGRP1 and RasGEF1B, which positively affects Ras activity. Thus E2F1 induces two positive feedback loops for survival

phosphorylates pRb to release E2F1. Inhibition of MAPK/ERK signaling caused the activation of pRb tumor suppressive activity and suppressed E2F1 and E2F3 activity suggesting the influence of this signaling on E2F1 activity in melanoma cells.[52] The crosstalk between ERKs and E2F1 is not unidirectional. E2F1 also regulates ERK activation via a transcriptional mechanism. E2F1-mediated transcription of RasGRP1 and RasGEF1B positively affects Ras activity. Thus E2F1 induces the Ras–Raf–MEK–ERK pathway. Altogether, the evidence thus far indicates an E2F1-ERK positive feedback loop that increases both ERK signaling and E2F1 activity.[53] This also suggests the existence of an alliance between E2F1 and ERK in promoting melanoma progression considering that there is overexpression of E2F1 and activated ERK signaling in malignant melanoma. Moreover, p73 a downstream target of E2F1 is also reported to form a positive feedback loop with oncogenic Ras. Sustained activation of Ras contributes to the stabilization of p73, which in turn ensures continuous activation of the MAPK cascade.[54] Deciphering the underlying mechanism could lead to the development of strategies to overcome E2F1-mediated inhibition of apoptosis and drug resistance.

Interaction with PI3K–AKT signaling. The PI3K–AKT signaling pathway is an emerging therapeutic target in malignant melanoma. Loss of heterozygosity of PTEN has been reported in approximately 30% of human melanomas.[55] However, PTEN loss is not the only reason for activation of this signaling. Oncoprotein Ras activates both the MAPK and PI3K–AKT pathways. Other activating mutations in melanoma including c-KIT, KIT receptors, ERBB4[56] may also contribute to activated signaling through this pathway. Studies using cultured melanoma cells and patient tumors have shown deregulated PI3K–AKT pathway activity in about 70% of melanomas.[57] Inhibition of the PI3K–AKT–mTOR signaling pathway potently sensitizes melanoma cells to chemotherapy with cisplatin and temozolomide.[58] Moreover, $BRAF^{V600E}$ serves as a negative regulator of the AKT pathway in melanoma, which may be responsible for the underlying molecular resistance mechanisms for BRAF inhibitors.[59] Chaussepied and Ginsberg[60] reported the existence of a negative feedback loop between E2F and AKT. Their work showed that the adaptor protein Grb2-associated binder 2 (Gab2) is a direct E2F1 target involved in this process. E2F1 induces AKT phosphorylation and activity by transcriptionally upregulating Gab2[60] and AKT suppresses E2F-induced apoptosis.[61] In this negative feedback loop, E2F1 promotes sustained AKT activation through Gab2, whereas AKT inhibits E2F1-mediated apoptosis. Topoisomerase II β-binding protein (TopBP1), a BRCA1 carboxyl-terminal (BRCT) domain-containing protein, also participates through AKT-mediated phosphorylation of TopBP1 at Ser-1159, which induces oligomerization of the protein.[60] This oligomerization through the seventh and eighth BRCT domain is required for E2F1 binding. Meanwhile, it prevents TopBP1 recruitment to chromatin and subsequent binding to ATR and hampers TopBP1 function in checkpoint activation.[62] TopBP1 interacts with and represses E2F1 only but not other E2Fs through its sixth BRCT domain.[63–65] As E2F1 is overexpressed in melanoma, this E2F1-Gab2-AKT-TopBP1-E2F1 feedback loop may explain why AKT is activated and E2F1 cannot exert its proapoptotic functions in malignant melanoma. In this way, targeting AKT pathways in melanoma may also remove constraints on E2F1 to induce apoptosis and could serve as a means to improve sensitivity of melanoma cells to AKT inhibitors.

Interaction with micro-RNA. MicroRNAs (miRNAs) are a class of short noncoding RNAs that regulate genes by directly promoting mRNA degradation or by repressing translation. They have important roles in proliferation and apoptosis, and are thus involved in the development of many cancers including melanoma. Expression of miR-205 is significantly suppressed in primary and malignant tumors when compared with nevi, and is correlated inversely with melanoma progression.[66,67] Several published reports strongly suggest that miR-205 might be a tumor suppressor and prognostic factor in melanoma, as its ectopic expression can inhibit melanoma cell growth and migration, and low level

of miR-205 is related to decreased disease-free survival of melanoma patients.[67–70] An interesting and complicated regulatory loop exists between E2F1 and miR-205. Dar *et al.*[66] found an inverse correlation between the expression of miR-205 and E2F1 and E2F5. By targeting the 3′UTR of these two E2Fs, miR-205 negatively regulates the Akt pathway. Overexpression of miR-205 inhibits cell proliferation, colony formation, and tumor growth and also induces apoptosis and senescence. Importantly, these phenotypes caused by miR-205 overexpression can be rescued by E2F1 overexpression. These data indicate that miR-205 works as tumor suppressor by repressing E2F1 and its gene targets. Downregulation of miR-205 may be responsible for the elevated level of E2F1 in primary and malignant melanoma.[66] The cause and effect relationship between E2F1 and miRNA is not simple as discussed above because E2F1 also downregulates miR-205 upon genotoxic stress, which may contribute to anticancer drug resistance.[71] E2F1 is known to induce expression of p73 and its N-terminally truncated isoforms (DNp73) via direct transactivation, which has a similar role in apoptosis induction like its homolog p53. DNp73 has antiapoptotic activity in human melanoma cells by either directly obstructing DNA binding or forming inactive heteromeric complexes with p73. p73 strongly induces miR-205, whereas the inhibitory DNp73 transdominantly inhibits it. E2F1 deficiency leads to DNp73 downregulation with a concomitant rise in miR-205.[71] This provides a possible explanation for the low miR-205 levels in the presence of high E2F1 activity in melanoma, that is, E2F1 downregulates miR-205 through stimulating DNp73 expression.

Potential Therapeutic Role for E2F1 in Melanoma

Despite the development of small molecule inhibitors for targeted therapy and immunotherapy for melanoma patients, a standard of care that can be applied to all melanoma patients is still missing. With none of the current approved drugs being curative, another challenge is to overcome therapeutic resistance. To improve therapeutic benefit and extend disease-free survival, combination therapies are currently being investigated. Although E2F1 may not be a good target *per se* considering the *Yin* and *Yang* biological effects it exerts, it may have applications in combination therapies and or serve as biomarkers.

Combination with chemotherapy. Given the resistance of malignant melanoma to conventional chemotherapy, would it be possible to sensitize drug resistant cells to apoptosis by manipulating E2F1 considering its role in apoptosis induction. In this regard, adenoviral vectors that express E2F1 (Ad-E2F1) efficiently induce apoptosis in cancer cells with little effect on normal cells.[72] Work by Dong *et al.*[73] showed that adenovirus-mediated E2F1 overexpression sensitizes melanoma cells to apoptosis induced by topoisomerase II inhibitors, like etoposide, and adriamycin with antitumor effects occurring *in vivo*. Later, Ad-E2F1 and doxorubicin combination treatment was found to produce synergistic effect on melanoma cell apoptosis by induction of antitumor cytokines, IL-8 and GM-CSF and inactivation of NF-κB pathway.[74,75]

Despite the promise of combinatorial use of E2F1, its controversial oncogenic role has led to the therapeutic testing of truncated E2F1 gene (E2Ftr; amino acids 1–375). Several studies have shown that mutants of E2F1 without TAD can induce cell death with as few as 75 amino acids within the DBD being sufficient for cell death.[76] Removal of TAD also hindered cell cycle-promoting activity and potently induced cancer cell apoptosis.[77] It is suggested that apoptotic effects of E2F1 are at least partly independent of its transactivation function as E2Ftr binds to promoters of prosurvival genes such as MCL1 without transactivation, and prevents wild-type E2F1 binding.[77] Therefore, understanding mechanisms that regulate E2Ftr-induced apoptosis can provide insight into the use of E2Ftr for melanoma therapy. BH3-only protein HRK is a possible target of E2F1, independent of its transactivation function. E2Ftr co-localizes with the HRK repressor downstream regulatory element antagonist modulator (DREAM) and promotes its homodimerization, to reduce DREAM binding to HRK promoter. However, the downregulation of HRK cannot completely repress E2Ftr-induced apoptosis, which suggests that there may be other pathways or factors involved in the apoptotic process.[12] The induction of apoptosis by E2Ftr is independent of p53 status with little cytotoxicity in normal cell lines. In a mouse melanoma xenograft model, overexpression of E2Ftr strongly induced caspase-3 activation with $\sim$80% decrease in tumor size.[78] Despite the potential benefits of E2Ftr obstacles such as effective ways of viral delivery, hepatic and other potential toxicity, and immune response against the adenovirus have to be overcome to realize its clinical utility.[78]

Potential role in drug resistance. A melanoma-specific explanation for drug resistance is melanosome-mediated sequestration of cytotoxic drugs that increases drug export.[79] For example, methotrexate (MTX) is exported out of resistant cells, so it cannot increase E2F1 protein levels as it does in sensitive cells. However, low intracellular MTX induces E2F1 demethylation, its acetylation and activation. Increased transcriptional activity upregulates downstream targets that are required for G_1 progression and prevents dTTP depletion in melanoma cells.[80] The accumulation of dTTP promotes DNA single-strand breaks and the subsequent activation of Chk1 to arrest cells in S phase and protect from apoptosis. Further, excess dTTP inhibits E2F1-mediated apoptosis in melanoma cells.[80] Interestingly, combination treatment with UCN-01 suppresses MTX export and promotes E2F1 apoptotic pathway.[81] In addition, the combination of MTX and tyrosinase-processed antifolate prodrug, 3-O-(3,4,5-trimethoxybenzoyl)-(-)-epicatechin (TMECG) causes depletion of thymidine pools, double-strand DNA breaks, and E2F1-mediated apoptosis with high efficiency regardless of BRAF, MEK, or p53 status. This is thought to be because MTX induces microphthalmia-associated transcription factor expression, which inhibits invasiveness

and promotes differentiation-associated expression of melanocyte-specific tyrosinase gene. Activation of TMECG generates TMECG-QM, which inhibits dihydrofolate reductase with high affinity, promotes dTTP depletion; S phase-associated DNA damage and E2F1-mediated apoptosis.[82] Together, these studies suggest that E2F1 is involved in the drug resistance of malignant melanoma, and it may be possible to overcome this resistance with the use of specific drug combination. Therefore, devising an optimum strategy that can affect E2F1-mediated apoptosis to overcome resistance is an area that deserves more attention.

Future Directions

The constant need for cancer cells to feed, divide, and grow lends credence to the idea that there is an unvarying need for transcription in cancer cells. Melanoma cells like most cancer cells are addicted to transcription factors including ATF-2, SNAIL/SLUG, NF-κB, STAT3, STAT5, E2F1, and others, many of which are known to be oncogenic. Transcription addiction allows cancer cells to meet their demand for gene products to enable their ability to proliferate, survive, migrate, invade, form new blood vessels, and so on. The involvement of E2F1 in cell cycle progression, proliferation, DNA damage response, and apoptosis have been known for many years. Recent evidence shows that E2F1 serves as a lever to regulate oxidative metabolism by switching from oxidative to glycolytic metabolism under stressful conditions.[83] As discussed in this review, E2F1 also affects migration and invasion of cancer cells through its interactions with signaling pathways to enable these functions. It would appear to be in the best interest of the melanoma cells to rely on a versatile transcription factor that can supply as many gene products as necessary for its growth and survival. In this regard, E2F1 is a useful candidate transcription factor for melanoma cells to be addicted to. Melanoma cells ensure their dependence on E2F1 through the deregulation of pRb-mediated negative regulation of E2F1. Abundance of E2F1 allows these cells to drive regulation of genes involved in many of the aforementioned biological processes. The draconic ability of E2F1 to control numerous signaling pathways directly or indirectly through its interactions and crosstalk between these pathways is prone to inhibit E2F1's apoptotic properties, promote its oncogenic activities, and lead to the observed activities associated with melanoma progression and drug resistance. However, recent observations including our unpublished observations suggest that this oncogenic view of E2F1 may be dictated differently in the context of mutations in the signaling pathways and produce an outcome that is in stark contrast to its so-called 'pro-cancerous' role. Further, as discussed here, E2F1 also binds directly to the hTERT promoter to repress c-MYC-mediated tumorigenesis. Given the contextual nature of E2F1's influence on various biological processes in normal and cancer cells we have to exercise caution in severing the arms of the E2F1 dragon so that it can be tamed in a way so as to restore its activities associated with death or senescence of cancer cells without activating other pathways involved in oncogenesis. In this regard, greater understanding of the changing biology of E2F1 especially in the context of cancer-specific mutations is warranted to ensure that E2F1 activity can be leveraged for the benefit of melanoma patients.

Conflict of Interest

The authors declare no conflict of interest.

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