The transcription factor E2F2 regulates tumor development

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ABSTRACT

Cell cycle-related E2F transcription factor 2 (E2F2) is a member of the E2F transcription factor family. E2F2 is implicated in tumorigenesis as it functions as a nuclear transcription factor responsible for regulating cell cycle progression to maintain genomic integrity. In recent decades, substantial experimental evidence has associated the function of E2F2 with tumor promotion. However, this transcription factor has also been revealed to be capable of exerting tumor suppressive activities in certain tumor models. In this review, we discuss recent developments in research that focus on E2F2 with respect to tumorigenesis. We will particularly address the dynamic regulatory roles of microRNA (miRNA) on the expression of E2F2 as various miRNA species have been confirmed to have complementary binding sites in the 3´ untranslated region of E2F2.

Keywords: E2F2, tumor, miRNA, transcription factor

OVERVIEW OF E2F2

In 1986, Kovesdi and co-workers discovered a group of transcription factors which interact with the ad−enovirus E2 promoter, an E1A inducible promoter from the nuclear extracts of adenovirus-infected cells[1]. The first family member, named E2F1, the gene product of which demonstrates the properties of E2F, was re−ported by Helin *et al*. in 1992[2]. Next, another novel protein in this family, E2F2 was cloned by Ivey-Hoyle *et al*. in 1993 by screening a HeLa cDNA library with a DNA probe derived from the DNA-binding domain of E2F1[3]. E2F2 and E2F1 exhibit 46% amino acid identity[4]. E2F2, which contains 10 exons coding for 438 amino acids, maps to 1p36.12 on the short arm of human chromosome 1. Since then 6 additional E2F family members with a high degree of homology have been discovered, and up to date altogether 8 homologs,

namely, E2F1 to E2F8, have been described[5]. They share a number of evolutionarily conserved domains, including a highly conserved DNA-binding domain, a dimerization domain which determines their interac−tion with differentiation-regulated transcription fac−tor proteins (DP), and an acidic amino acid-enriched transactivation domain within which a tumor suppres−sor protein association domain is embedded.

E2F2 is an important member of the E2F family of transcription factors that are capable of exerting regulatory roles in dynamic cellular processes includ−ing differentiation, development, proliferation and apoptosis[6]. E2F2 functions as a nuclear transcription factor responsible for regulating mitosis to maintain genomic integrity. Activation of E2F2 promotes the G1-to S-phase transition in cell cycling, indicating that this molecule is one of the key regulators in cell cycle progression. Recently, substantial experimental evi−dence has associated the function of E2F2 with tumor

promotion in addition to many physiological processes regulated by this transcription factor.

Studies resolving the interactions between E2F transcription factors and retinoblastoma protein (pRb), a tumor suppressor protein, have contributed to the elucidation of the common function of E2F proteins. pRb is the founding member of a protein family of tumor suppressors that demonstrates a crucial role in the regulation of G1-to S-phase transition. Loss of its control over cell cycle progression is one of the mechanisms that account for tumorigenesis[7]. These proteins fold into a binding pocket-like structure and are thus collectively addressed as the pocket protein family[8]. pRb is capable of regulating entry into S−phase through a direct interaction between its large pocket domain and the transactivation domain of E2F members (e.g., E2F1, E2F2, and E2F3) resulting in the repression of E2F-dependent transcription of S−phase-promoting genes including DNA polymerases. In G0 and early G1, pRb physically interacts with E2F factors, thereby blocking the transactivation domain of E2F. At the onset of S-phase, E2F is released from the pRb-E2F complex as a result of phosphorylation of pRb which is driven by cyclin D and its associated cyclin-dependent kinases (CDK). Dissociation of E2F from pRb exposes E2F-binding sites for the promoters of cell-cycle proteins, resulting in cell cycle progres−sion[7,9]. Therefore, E2F, through collaboration with pRb, is required for the full execution of cell cycle progression and this family of transcription factors is involved in the regulation of tumor development.

Loss of E2F2 is generally correlated with decreased proliferation of tumor cells, which is consistent with its role in promoting the transcription of quite a num−ber of cell cycle-promoting genes. Paradoxically, de−pending on the context, mice with mutations in E2F2 can exhibit increased cancer incidence[10,11]. This para−dox is also reflected in certain human cancers, such as in human glioma[12], breast cancer[13], colon cancer[14], and gastric cancer[15], whereby it is revealed that E2F2 exerts tumor suppressive or prognostic activities. We will discuss these models in further detail below.

Interplay between microRNA (miRNA) and E2F members including E2F2 has been an attractive area of research in tumor biology. miRNAs are a family of 21-to 25-nucleotide, noncoding small RNAs that pri−marily function as gene regulators. Elucidation of the roles of miRNAs in tumorigenesis has advanced our understanding in the complex mechanisms of cancer development in recent years[16,17]. It is widely ac−cepted that altered miRNA expression impacts tumour growth, progression and metastasis. In this review, we will also discuss the interaction between miRNA and

E2F2 as well as the functional consequence of this in−teraction in a number of tumor models. In addition to regulating cell cycle progression, pRb has been impli−cated in various cellular events, including differentia−tion, apoptosis, motility, cartilage development, and vascularization[18]. Likewise, E2F proteins, including E2F2, also exert dynamic regulatory roles in other pathophysiologic settings in addition to cancer, which is not the focus of this review.

THE REGULATORY EFFECTS OF E2F2 ON TUMOR DEVELOPMENT

Leukemia

Leukemia collectively refers to hematopoietic stem cell-initiated heterogeneous blood cancers. Leuke−mia usually originates in the bone marrow and re−sults in abnormal blood cell proliferation in both the bone marrow and peripheral blood. The uncontrolled growth of blood cancer cells outgrow normal blood cells causing symptoms that include easy bleeding or bruising, persistent fatigue, frequent fevers, and an increased risk of infections. Leukemia is grouped by how rapidly the disease develops, i.e., acute or chronic, as well as by the major type of blood cells involved, i.e., lymphocytes or myelocytes. Thus, leu−kemia is mainly divided into four main types: acute lymphoblastic leukemia (ALL), acute myeloid leu−kemia (AML), chronic lymphocytic leukemia (CLL), and chronic myeloid leukemia (CML)[19]. The most common types of leukemia in adults are AML and CLL, followed by CML and ALL. Despite the fact that approximately 10 times more adults than children de−velop leukemia, it is the most prevalent cancer among children, with ALL accounting for approximately 75 percent of all childhood leukemia cases. The overall yearly incidence for leukemia has increased modestly in recent decades, while overall mortality rates have fallen since the 1990s[20].

Increase in steady-state E2F2 protein is correlated with the level of Tax protein of human T-cell leukemia virus type Ⅰ, a transcriptional activator implicated in cel−lular transformation and leukaemia development[21,22]. The requirement of myeloid translocation gene 16 (MTG16) for maintenance of haematopoietic stem cell quiescence is probably associated with its capacity to suppress the expression of a number of key cell cy−cle regulators including E2F2[23]. Although enhanced leukemogenesis is observed in E2F1/E2F2 double knockout mice, this is found to be primarily due to the weakened capacity of double knockout progeni−tor cells to compete with Bcr-Abl-expressing double knockout progenitors rather than cell autonomous ef−

fects of E2F2 loss [24]. In conclusion, experimental ev‐idence supports the cancer promoting effect of E2F2 in leukemia. However, the regulatory role of E2F2 in leukemia has not been thoroughly investigated. Therefore, this is an area that awaits further experi‐mental exploitation and input.

Glioma

Glioma refers to a type of tumor that starts in the glial cells of the brain or spinal cord. Glioblastoma, also known as glioblastoma multiforme (GBM), is the most frequent, aggressive and deadly primary malig‐nant tumor of the central nervous system, accounting for 60%~70% of all gliomas and 15% of all intracra‐nial tumors. No satisfactory treatment for this fero‐cious type of malignancy is available, and the median survival time is less than 16 months following initial diagnosis [25]. Management of glioblastoma remains palliative as optimal treatment including surgery and chemotherapy only results in a median survival of less than one year. Only about 2% of patients survive three years.

Network-based survival analysis reveals that high expression of E2F2 is correlated with poor prognosis. By exploiting data in the Chinese Glioma Genome Atlas (CGGA) database, Zhang *et al*. found that ex‐pression levels of E2F2 in glioma are higher than normal brain tissues, and miRNAs play crucial roles in regulating the tumorigenesis of glioma. Further‐more, they revealed that miR-218 inhibits the growth and metabolism of gliomas by directly targeting E2F2. Reduced expression levels of miR-218 are observed in human glioblastoma tissues. In contrast, enforced expression of miR-218 in human glioblastoma cell lines arrests cell cycle progression in the G1-phase, thus profoundly suppressing cell proliferation. Both glucose consumption and lactate production by glio‐blastoma cells are also inhibited with enhanced ex‐pression of miR-218. It is thus proposed that miR-218, a repressor of E2F2 in glioblastoma, may be exploited as a therapeutic target [26]. Two binding sites for miR‐125b, which inhibits the proliferation of glioblastoma stem cells, are identified in E2F2, suggesting the in‐volvement of E2F2 in miR-125b-mediated regulation of glioblastoma development [27]. Low expression of PPARα is correlated with poor prognosis of glioma and GBM patients, and the mechanism may be as‐sociated with PPARα-induced expression of miR‐214 which suppresses its target E2F2 [28]. One more piece of evidence showing the regulation of E2F2 by miRNA in glioma cells comes from the study demon‐strating that the Let-7 miRNA precursor inhibits the malignant development of glioma cells via down-reg‐ulation of E2F2 [29]. In contrast to the tumor promoting activity of E2F2, it is also observed that enforced ex‐pression of E2F2 enhances p53-mediated anti-cancer effect in human glioma cells [12].

Lung cancer

Being the most common cause of cancer deaths worldwide, lung cancer is one of the most aggressive tumors, with very low life expectancy. Lung can‐cer includes two broad histological subtypes: small‐cell lung cancer (SCLC), which is the cause of 15% of cases, and non-small-cell lung cancer (NSCLC), which accounts for 85% of cases. NSCLC is further divided into adenocarcinoma, squamous-cell carci‐noma, and large-cell carcinoma [30]. Surgery remains the standard of treatment for early-stage NSCLC. However, not all patients benefit from tumor resection by surgery as some patients develop recurrence after surgery. Biomarkers that help determine clinical stag‐ing and predict treatment outcome are therefore war‐ranted.

As mentioned above, E2F2 promotes lung metas‐tasis in breast cancer [13, 31]. Chen *et al*. analyzed lung samples from 86 NSCLC patients by immunohis‐tochemistry as well as lung tissues from 8 NSCLC patients by Western blotting for E2F2 expression [32]. Both approaches consistently confirmed that E2F2 expression is markedly increased in NSCLC tissues compared with adjacent normal tissue controls. Fur‐thermore, E2F2 expression is correlated with tumor cell proliferation and poor patient prognosis. Of note, E2F2 expression is closely correlated with clinical stage and tumor size. Knockdown of E2F2 expres‐sion reduces the viability and colony formation ca‐pacity of NSCLC cells [32]. Based on exploitation of the Oncomine, GEPIA, Kaplan-Meier(KM) Plotter, and cBioPortal databases, Sun *et al*. made interesting observations regarding E2F family proteins [33]. Ex‐pression levels of E2F2, among a group of other E2F factors, are higher in lung adenocarcinoma and squa‐mous cell lung carcinoma tissues than in normal lung tissues. The expression levels of E2F2, among certain other E2F family members, are positively correlated with advanced tumor staging. High transcription lev‐els of a panel of E2F members including E2F2 are correlated with low relapse-free survival in all lung cancer patients. Taken together, these findings support E2F2, together with E2F1, E2F4, E2F5 and E2F8, as new biomarkers for the prognosis of lung cancer [33]. Based on bioinformatics analysis, E2F2 expression is found to be significantly correlated with NSCLC patient gender and tumor size, with higher expression levels in males over females and higher expression in

later stages. Furthermore, overexpression of E2F2 is correlated with poor prognosis [34]. Through analyzing miRNA expression in 47 lung cancer biopsies, Feliciano *et al.* reported that expression of miR-99a, which is capable of downregulating E2F2 by direct binding to the 3´ untranslated region (3´-UTR) of E2F2, is correlated to decreased proliferation, cell cycle arrest and apoptosis of a panel of lung cancer cell lines [35]. It is recently revealed that circular RNA circPVT1 promotes tumor proliferation and invasion through sponging miR-125b and activating E2F2 signaling in NSCLC [36], as E2F2 is a target of miR-125b which has a protective role in some cancers, e.g., glioblastoma as mentioned above [27].

Breast cancer

Breast cancer is the most common cancer in women worldwide and the second most common cancer overall after the lung cancer. Although steady increases in the incidence of breast cancer in western countries were recorded toward the end of last century, which is believed to be primarily due to increased screening, reproductive pattern changes, and wide use of menopausal hormonal therapy, breast cancer incidence has declined since the early 2000s [37]. However, breast cancer still accounts for more than 268,000 new cases and 40,000 fatalities in the US alone in 2019 [38]. Breast cancer incidence rates in less developed areas of the world continue to increase, probably reflecting changing reproductive patterns as well as increased screening [37].

Based on the datasets in Oncomine and the Cancer Genome Atlas, E2F2, together with a group of other E2F factors including E2F1, E2F3, E2F5, E2F7 and E2F8, is overexpressed at the mRNA level in patients with breast cancer [39]. Rennhack *et al.* found that loss of E2F1 or E2F2 leads to reduced metastasis in mouse mammary tumor virus (MMTV)-Neu mice which are prone to mammary tumorigenesis. They observed that 73% of the mice with mammary tumor induction developed metastasis to the lungs, which stands in clear contrast to the reduced percentage of 35% when E2F2 is deleted in similar mice. Importantly, the model is supported by analyzing human HER2-positive cancer patients [40]. In the mouse MMTV-polyomavirus middle T oncoprotein (PyMT) model of metastatic breast cancer, it is noted that deficiency in E2F2 strikingly reduces tumor metastasis [31]. It is reported that E2F2, with three binding sites, is a direct target of miR-4319 which is capable of suppressing breast tumor initiation and metastasis [41]. Breast cancer cell-matrix adhesion and outgrowth are profoundly inhibited when E2F2 levels are lowered [42]. Knockdown of E2F2 expression results in disruption of estrogen receptor-positive breast cancer cell proliferation [43]. Activating protein-1 (AP-1), a transcription factor that regulates gene expression in response to a variety of stimuli, is involved in promoting breast cancer cell growth, and suppression of AP-1 expression is correlated with reduced expression of E2F1 and E2F2 [44]. E2F2 deficiency suppresses breast cancer metastasis and hence results in reduced circulating tumor cells [45]. All these cases are consistent with the major role of E2F2 as a tumor promoter. However, studies pointing to the contrary have also been reported. Loss of E2F2 promotes lung metastasis in breast cancer, whereas downregulating the expression of the tumor suppressor gene PTPRD suppresses lung metastasis, suggesting antagonism between E2F2 and PTPRD [13]. It is also been revealed that genetic deletion of E2F2 accelerates mammary tumorigenesis driven by Myc-overexpression [46].

Gastric cancer

Gastric cancer develops from the lining of the stomach and remains one of the most common and deadly human neoplasms, with an estimated annual global death toll of up to 800,000 [47]. More than 70% of these cases occur in developing countries, and China is one of the countries with the highest incidences of gastric cancer, accounting for over 40% of all new gastric cancer cases in the world [48,49]. Most cases of stomach cancer are gastric carcinomas, which can be further divided into a number of subtypes including gastric adenocarcinomas [50]. Surgery remains the major choice of treatment for stomach cancer, supplemented with chemotherapy and radiation therapy [51]. Most gastric cancer patients are asymptomatic in the early stages which makes timely diagnosis difficult. Therefore, diagnostic markers which help to reveal early-stage asymptomatic cases are desperately needed.

miR-31 has been found reduced in gastric cancer and the expression of miR-31 is negatively correlated with poor tumor differentiation, lymph node metastasis, advanced tumor staging and worse overall survival of gastric cancer patients. Interestingly, E2F2 is the direct target of miR-31. Consistently, E2F2 expression, which is inversely correlated with miR-31 expression, is upregulated in gastric cancer tissues. Furthermore, knockdown of E2F2 expression mimics the antitumor activity of miR-31 in gastric cancer cells, while the ectopic expression of E2F2 can rescue miR-31-mediated inhibition in the growth of gastric cell lines. Taken together, these results demonstrate that miR-31 acts as a crucial tumor suppressor by inhibiting E2F2 expression [52]. miR-26a, which has a target site in the 3´-UTR of E2F2, is downregulated in

chemotherapy-resistant gastric cancer cells. Further–more, knockdown of E2F2 sensitizes gastric cancer cells to chemotherapy [53]. These results suggest that miR–26a might improve the sensitivity of gastric can–cer cells to chemotherapy through targeting E2F2.

Despite these models demonstrating the gastric cancer promoting activity of E2F2, the antitumor ac–tivity of E2F2 has also been observed. By using the KM Plotter database, which has been established us–ing gene expression data and survival information from the Gene Expression Omnibus (GEO), Manicum and co-workers [15] reported that increased expression of E2F2, together with E2F5, E2F6 and E2F7, is cor–related with favorable overall survival, especially for late-stage gastric cancer patients. These results pro–vide insight into the controversial prognostic function of E2F in gastric cancer. Although the results should be further verified in clinical trials, these findings suggest E2F2 may be a favorable prognostic predictor for evaluating the efficacy of therapeutic drugs in the treatment of gastric cancer.

Other malignancies

Evidence for the involvement of E2F2 in the regu–lation of other types of cancers has also been steadily accumulated. Chen *et al*. reported that E2F2, a target of miR-365, is highly expressed in cholangiocar–cinoma tissues and cell lines, whereas miR-365 is downregulated in cholangiocarcinoma tissues com–pared with adjacent normal tissues. In an experimental model, overexpression of miR-365, which suppresses the expression of E2F2, is capable of inhibiting chol–angiocarcinoma cell proliferation and drives apoptosis of these cancer cells. This finding is consistent with the clinically observed inverse correlation between the expression levels of E2F2 and miR-365 in cholangio–carcinoma tissues. These data suggest that the inhi–bition of E2F2 activity may benefit the treatment of cholangiocarcinoma [54]. Similar to the protective role of miR-218 revealed in glioma [26], it is also reported that overexpression of miR-218 and miR-520a can inhibit proliferation of hepatocellular carcinoma cells. miR-218 and miR-520a are observed to negatively regulate E2F2 expression despite the fact that E2F2 is a direct target of miR-218, but not miR-520a. These results indicate that E2F2 is a critical tumor promoting molecule via which miR-218 and miR-520a regulate the development of hepatocellular carcinoma directly or indirectly, respectively [55]. Another miRNA, miR–490-5p, has also been recently implicated in the sup–pression of hepatocellular carcinoma through down–regulating E2F2 expression [56].

Differentiation antagonizing nonprotein coding RNA (DANCR), a type of long non-coding RNA (lncRNA), is profoundly upregulated in pancreatic cancer tissues as DANCR functions as a molecular sponge for miR-214-5p that inhibits E2F2 expression [57]. It is reported that human papillomavirus (HPV)16 E6 oncoprotein is capable of downregulating miR-496, which targets the 3´ UTR of E2F2, providing a mech–anism which underlies the role of HPV16 infection in oropharyngeal cancer [58]. Lower expression levels of miR-125a are observed in osteosarcoma tissues and miRNA-125a is found to be able to regulate tumor cell proliferation via targeting E2F2 [59].

Two more examples supporting E2F2 as a tumor suppressor come from studies addressing colon cancer and clear cell renal cell carcinoma. E2F2 is shown to inhibit the growth and proliferation of colon cancer cell lines [14]. In this study, it is worth pointing out that miR-31, which can directly target E2F2, promotes the proliferation of colon cancer cells. This stands in clear contrast to the above mentioned miR-31-mediated tumor suppression in gastric cancer [52]. Gao *et al*. re–ported that miR-155 functions as a tumor-enhancer by suppressing E2F2 in clear cell renal cell carcinoma, suggesting the tumor suppressive effect of E2F2 in this renal malignancy [60].

SUMMARY AND PERSPECTIVES

As a member of the E2F family of transcription factors, E2F2 plays a critical role in the regulation of cell cycling, which has a profound impact on tumor initiation and growth. The potential of E2F2 in regu–lating tumor development is dynamic and context–dependent. In different cancers or during different tumor developmental stages of the same cancer, this transcription factor may exert distinct functions. Of note, E2F2 not only has many downstream targets for initiating gene expression, thereby modulating tumor development, but also is subject to the regulation of various upstream signals such as pRb and miRNA (***Fig.1***). The enigmatic roles of E2F2 on the promo–tion and suppression of different tumors await further clarification. It is possible that certain non-canonical roles of E2F2, i.e., activities other than cell cycle regulation, have not been revealed. Future research is warranted to fill the gaps in our understanding of the precise regulatory mechanisms of E2F2 as well as to elucidate the complex relationship between E2F2 and other E2F family members and tumor regulatory molecules. Targeting E2F2 may facilitate the rational design of cutting-edge diagnostic tools with better clinical relevance and novel anticancer therapies with higher efficacy.
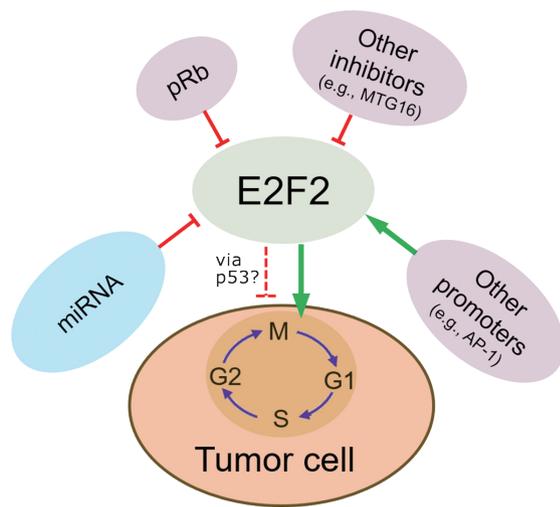
Fig. 1 Regulation of tumor development by E2F2 and associated partners. This schematic diagram illustrates the possible interactions between the transcription factor E2F2 and its regulatory partners for impacting tumor development. Overwhelming experimental evidence suggests that E2F2 promotes tumor development by stimulating cell cycle progression (green pointed arrow). However, some studies suggest an opposite, suppressive effect of E2F2 on tumor cells (red dashed blunt arrow), possibly via enhancing p53-mediated anti-cancer effect (ref 12). Various microRNA (miRNA) species, which have been confirmed to have complementary binding sites in the 3´ untranslated region of E2F2, are capable of exerting inhibitory effects on E2F2(red blunt solid arrow). E2F2 is also subject to an inhibitory regulation by other molecules such as retinoblastoma protein (pRb) and MTG16 (red blunt solid arrows). Factors (e.g., AP−1) that may upregulate E2F2 have also been reported (green pointed arrow). G1-S-G2-M, respective cell cycle phases; MTG16, myeloid translocation gene 16; AP−1, activating protein-1.

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