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Review

Promising roles of mammalian E2Fs in hepatocellular carcinoma

Lei Zhan, Cheng Huang, Xiao Ming Meng, Yang Song, Xiao Qin Wu, Cheng Gui Miu, Xiang Shu Zhan, Jun Li *

School of Pharmacy, Anhui Medical University, Meishan Road, Hefei 230032, China
Institute for Liver Diseases of Anhui Medical University (AMU), China

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ABSTRACT

In mammalian cells, E2F family of transcription factors (E2Fs) traditionally modulates assorted cellular functions related to cell cycle progression, proliferation, apoptosis and differentiation. Eight members, E2F1 E2F8 have been recognized of this family so far, and the members of this family are generally divided into activator E2F (E2F1––E2F3a), repressor E2F (E2F3b––E2F5) and inhibitor E2F (E2F6––E2F8) subclasses based on their structur–e and function. Studies have showed that the mammalian E2F family members represent a recent evolutionary adaptation to malignancies besides hepatocellular carcinoma (HCC), and a growing body of evidence has validated that the individual members of the family develop a close relationship with HCC. E2F1 was identified to play overlapping roles in HCC, while E2F2––E2F8 (except E2F6 and E2F7) showed to be tumor-promoter in HCC. However, the mechanism underlying the mammalian E2Fs associated with HCC is still unknown and needs further research. The aim of this review is to sum up the collective knowledge of E2F family and the roles of each member of this family in HCC. Moreover, we will discuss some novel therapeutic target for HCC based on the complicated functions of mammalian E2Fs.

Contents

1. Introduction

Hepatocellular carcinoma (HCC)is the sixth most commonly occurring cancer and the third leading cause of cancer-related deaths every year worldwide [1,2]; despite a mass of researches aimed at HCC have been carriedout in the past years, the precise molecular mechanism involved in HCC is still poorly understood and even worse, the overall incidence of HCC is still increasing [3]. Recently, mammalian E2F family of transcription factors is identified to have a close relationship with HCC, which may help us develop novel therapeutic approaches for HCC.

The mammalian E2Fs locate in the downstream of the cell cycle signaling cascades, and they turned out to be playing vital roles in cell proliferation, apoptosis, differentiation, senescence, DNA-damage

Abbreviations: E2Fs, E2F family of transcription factors; HCC, hepatocellular carcinoma; pRb, retinoblastoma protein; CDK, cyclin dependent kinase; DBD, DNA binding domain; DP, dimerization partner; LZ, leuzine zipper; MB, marked box; NLS, nuclear localization signal; CycA, cyclin A-binding site; NES, nuclear export signals; PcG, Poly-comb group; DIM, dimerization; CCND1, cyclin D1; DHFR, dihydrofolate reductase; TGB, target gene bias analysis; ChIP, chromatin immunoprecipitation; TGF-β, transforming growth factor-β; AML, acute myeloid leukemia.

* Corresponding author at: School of Pharmacy, Anhui Medical University, Meishan Road, Hefei, Anhui Province 230032, China. Tel./fax: +86 551 65161001.
E-mail address: lj@ahmu.edu.cn (J. Li).

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response and DNA repair through modulating the genes which correlate with cell cycle progression in mammalian cells [4–6]. E2F was first identified as a cellular factor required for the activation of the E2 adenoviral promoter in the middle 1980s [7], and later named E2F1 which is a classic member of E2Fs. To date, E2F3b, E2F7 and E2F8, as the new members of E2Fs "appear", eight members of this family have been recognized, E2F1––E2F8, coding at least nine different E2F forms [8]. E2Fs, associate with the retinoblastoma protein (pRb) and the pRb-related proteins p107 and p130 which are regulated by cyclin dependent kinases (CDKs), form the CDK––E2F–Rb network and take part in the transcriptional activities [9]. As the researches continue, we find that the activities attributed to E2Fs have become more complicated [5]. The study of mammalian E2Fs has been taken into account because they play key cancer-related roles through their essential features to regulate a variety of cellular functions [10–12]. Evidence has showed that E2F1 and E2F3 are significantly over-expressed in HCC compared to non-tumor liver tissues [13,14]. Furthermore, E2F2 has been proved to associate with breast cancer [15]. In addition, E2F7 and E2F8 were critical regulators of angiogenesis [16,17] and E2F4 related to micro-satellite unstable gastric adenocarcinomas during tumor progression [18]. Noteworthily, accumulated evidence has indicated that the E2Fs had intimate connections with HCC in recent years [19–21].

In this review, we intend to sum up the collective knowledge of E2Fs firstly, and then we focus on the potential roles of each member of mammalian E2F family in HCC,further providing novel therapeutic approaches for HCC.

2. Category and structure of E2Fs

The mammalian E2F family is conventionally classified into the following three categories based on their functional properties as well as structural features [22]. (i) Activator E2Fs (E2F1––E2F3a), they are transcriptionally activators of E2F target genes, and they regulate the transcriptional activities by binding to the promoters of target gene via their response elements during the cell cycle progression of late G1/S phase [23]. (ii) Repressor E2Fs (E2F3b––E2F5), they constrain the transcription of E2F target genes when cooperate with E2F inhibitory pocket proteins and with the addition of repressive histone deacetylases during the early G1 phase [6,24]. This subclass keeps geostationary throughout the whole cell cycle progression [25]. (iii) Inhibitor E2Fs (E2F6––E2F8), this group of E2Fs shares similar functions with "repressor E2Fs" but not via interaction with pocket proteins [26–28]. Specifically, E2F3 has two highly related isoforms, E2F3a and E2F3b, which are transcribed from distinct promoters [29], while the two isoforms of E2F7, E2F7a and E2F7b are produced by alternative splicing of the primary transcript [26]. Nevertheless, it should be emphasized that the basic classification of mammalian E2Fs is generally depending on the in vitro study and lack of in vivo identification, so the elegant classification may not be sufficient to highlight their sophisticated roles [5,8,23,30].

The mammalian E2Fs can also be broadly sub-divided into typical E2Fs (E2F1––E2F6) and atypical E2Fs (E2F7––E2F8) based on their signature winged-helix DNA binding domain (DBD) [31]. All members are expressed from eight chromosomal loci which encode nine distinct gene products [8]. Typical E2Fs carry one evolutionary conserved DBD, and bind DNA as heterodimers with one of three dimerization partner (DP) proteins, DP1, DP2 and DP3. Furthermore, this heterodimerization is modulated by the leuzine zipper (LZ) and marked box (MB) domains [15]. Notably, E2F6 possesses a C-terminal transactivation domain containing the binding region which proved to bind with Poly-comb group (PcG) [27]. In mammalian cells, most typical E2F DNA binding comes up once E2F––DP heterodimers form [32]. Different from typical E2Fs, the atypical E2Fs consist of a separate and highly evolutionarily conserved repressor arm and their isoforms are produced by alternative splicing of the primary transcript [26]. In addition, the DBD is duplicated in atypical E2Fs and they bind to the promoters of target gene in a DP-independent mode owing to the deficiency of DP- binding domain [26,28], yet the second DNA-binding domain is likely to substitute for the function of the DP subunit in DNA binding. Moreover, the atypical E2Fs also lack a recognizable transactivation domain or pocket protein binding domain compared to typical E2Fs [33]. The molecular features of typical E2Fs and atypical E2Fs are shown in Fig. 1. Maybe we get the different molecular structure between typical and atypical E2Fs, but up to now, it is still undefined whether atypical E2Fs resulted from the gene duplication of typical E2Fs or whether the typical category lost a second DBD during evolution and more studies are needed in the future.

3. E2Fs transcriptional regulation

As referenced, the mammalian E2F family plays a significant role in regulating cell cycle, proliferation, apoptosis as well as other cellular processes [6]. It is noticeable that "Rb pathway" is one of the most significant pathways in normal cell cycle control. Intriguingly, the E2Fs are well demonstrated as a primary transcriptional regulator controlled by the Rb family [34], members of Rb family bind with the E2F transcription factors that cause negative regulation of E2F target genes. It is well demonstrated that the Rb family consists of pRb and the related pocket proteins p107 and p130 [35]. When Rb maintains in a certain state of hypophosphorylation, E2F1–E2F5 will bind to Rb in G0/G1 phase and form evolutionary conservation of Rb–E2F repressor complexes, leading to block transcriptional activation by E2Fs [36]. In light of this, due to the exclusive interaction of E2F1–E2F3 with pRb in the context of pRb hyperphosphorylation, which leads to the losing of pRb function,that results in a disruption of pocket protein–E2F complexes [10], E2F1–E2F3 dissociate from pRb and the deregulation of E2Fs makes entrance of quiescent cells in the S phase of cell cycle, further exerting pleiotropic regulation on cellular functions, that is to say, E2F1–E2F3 are expressed in a cell cycle-regulated manner and exhibit the highest levels in the late G1 and S phase [23]. By the way, the phosphorylation of pRb is stimulated by CDKs which are inhibited by $p16^{INK4a}$ [10]. Moreover, we find that ectopically expressed E2F1––E2F3 are localized to the nucleus owing to their amino-terminal nuclear localization signal (NLS) sequence, which is adjacent to the cyclin A-binding site (CycA) [37]. E2F4 and E2F5, as effectors of Rb family, E2F5 tends to connect with p130 while E2F4 can bind to any members of the Rb family [38]. In G0 phase, the ubiquitously expressed E2F4 and E2F5 associate with pocket proteins and other co-repressors to maintain repression of E2F-responsive genes, and promote entry into the G1 phase of the cell cycle. Consistently, the sequential phosphorylation of Rb family make E2F4 and E2F5 released then further predominantly occupy the E2F-regulated promoters in G0/G1 phase which was mediated by bipartite nuclear export signals (NES) [38]. E2F6, diverges considerably from the previous E2F subgroups, bears little homology outside the DBD and dimerization (DIM) domains and is presumed to repress E2F-responsive genes independently of Rb family, E2F6 has also been shown to repress transcription through association with PcG proteins [27]. Evidence illustrated that over-expression of E2F6 could repress the transcription of known E2F-responsive genes in vitro and it appeared to function as a dominant negative inhibitor through competition with other E2F family members [39]. As new players of the E2F family, E2F7 and E2F8 are unique because they form homodimers (E2F7––E2F7 and E2F8––E2F8) or heterodimers (E2F7––E2F8) to repress transcription of cell cycle-related genes, moreover, their transcription is induced at the G1-to-S transition reaching their peak during S-to-G2 transition [31]. It was noteworthy that, E2F7 and E2F8 were detected independently in a screen for E2F1 activities related to cell cycle traverse analogous to E2F6 [40].

From the above, it seems that repressor E2Fs (E2F4––5) occupy E2F promoters in G0 phases and early G1 phases, and recruit chromatin remodeling factors through binding with p107 and p130. As cells enter into late G1 phases, the repressor E2F complexes are replaced by