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Involvement of E2F transcription factor family in cancer

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Abstract

The E2F family of transcription factors is a central modulator of important cellular events, including cell cycle progression, apoptosis and DNA damage response. The role of E2F family members in various human malignancies is yet unclear and may provide vital clues to the diagnosis, prognosis and therapy of cancer patients. In this review we provide a brief but concise overview of E2F function and its putative role in the most common human tumour types.

Keywords: Transcription factors; Cancer; E2F transcription factors; E2F1 transcription factor; Cell cycle; Apoptosis; DNA damage; Lung cancer; Breast tumours; Bladder tumours

1. Introduction

Evolution has employed multiple redundant circuits that couple proliferation with apoptosis in order to protect the cell when proliferation becomes aberrant. In cancer this link is targeted. Understanding the role of transcription factors (TFs) in abnormal cellular settings is likely to promote the elucidation of the mechanisms leading to the molecular dissociation of proliferative and innate suppressive programs. In addition, based on this information more effective tumour-specific therapies can be designed [1,2].

A very important group of TFs, irrevocably linked with cell cycle control and apoptosis, is the E2F family. The founding member E2F1 was discovered in the mid 1980s as a transcriptional activator of the adenovirus E2 promoter [3,4] and hence its name. Subsequently, it became clear that E2F was bound to a cellular protein, now known to be pRb, and released by the viral E1A gene product [5]. Since then, the family has grown considerably and currently contains six established members and two newer ones. In this review, we attempt to shed light on the role of E2F in carcinogenesis, particularly, on its dual cellular behaviour in primary human malignancies.

2. The E2F family organisation

2.1. The E2F members

With the addition of two newer members, the E2F family consists of eight proteins in mammals. E2F1-6 are the older and better characterised members (Fig. 1), whereas E2F7 [6,7] and E2F8 (in mouse) [8] were only recently identified and bear little homology to their traditional counterparts. All the established members (E2F1-6) possess N-terminal DNA binding and dimerisation domains, followed by the marked box, a conserved region involved in dimerisation and DNA bending. With the exception of E2F6, they have a C-terminal transactivation domain which contains the pocket protein binding region. Other structural differences include the presence of nuclear localisation signals (NLS) [9] and cyclin A binding domains in the

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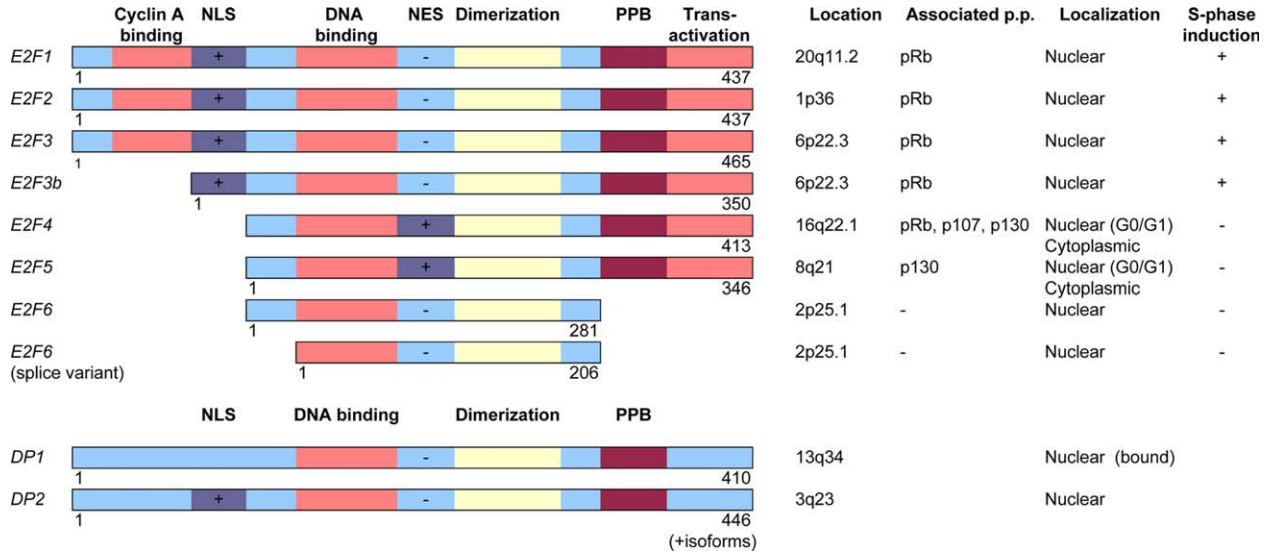

Fig. 1. The E2F family of transcription factors. This figure summarises the structural characteristics and key properties of E2Fs. Light blue characterises domains of unknown significance. NES, nuclear export signal; NLS, nuclear localisation signal; PPB, pocket protein binding domain (for details, see Sections 2.1 and 2.2).

N-terminal regions of E2F1-3. Notably, the E2F3 gene encodes two distinct protein products, with the shorter E2F3b form lacking the cyclin A binding domain [10]. The E2F4 and E2F5 proteins are tagged with nuclear export signals (NES) and rely on heterodimerisation with DRTF polypeptide (DP) members to ensure their translocation to the nucleus. The presence of localisation signals secures continuous nucleo-cytoplasmic movement which is important in modulating effectively the activity of E2Fs in a cell cycle-dependent manner [11].

Based on the differing homology, which apparently affects their function, the E2F family is divided into three groups. E2F1-3 comprise the activating group due to their ability to induce S-phase entry in quiescent cells [12–14] and overcome arrest mediated by the $p16^{INK4a}$ cyclin-dependent kinase inhibitor (CDKI) [15]. On the other hand, E2F4 and E2F5 constitute the repressor group which seems to be important for cell cycle exit and terminal differentiation processes, such as adipogenesis [16,17] and erythrocyte maturation [18]. Finally, E2F6 [19,20] although acting as a repressor, is unique due to its ability to interact with members of the mammalian polycomb complex (PcG) and not with the pocket proteins [21].

2.2. The E2F-DP heterodimers

Functional E2F depends on the formation of heterodimers with members of the DP family of transcription factors. The heterodimeric nature of E2F complexes was first reported by Girling *et al.* [22], who isolated DP-1 (**D**RTF-1 **P**olypeptide **1**). The second member of the DP family was later identified [23,24]. DP proteins have significant homology with E2Fs, sharing the dimerisation and DNA binding domains (Fig. 1). All E2F-DP combinations have been shown to exist *in vivo* [25]. The number of putative complexes is likely to be even greater due to mechanisms such as alternative splicing of the DP proteins [24,26].

The cooperation between E2F and DP is suggested to optimise DNA binding activity in a synergistic fashion [27,28]. Furthermore, the presence of a nuclear localisation signal in DP-2 and its absence from DP-1 may influence the accumulation of E2F in the nucleus [29,30]. DNA binding specificity is defined by the E2F subunit [25], whereas the role of the DP subunit in the E2F complex is less well understood. Nevertheless, the DP protein may enhance proliferation, as shown by *in vivo* models [31]. Additional studies are required to elucidate this point.

3. Regulation and function of the E2F family

Over the past years, the E2F family, especially the founding member E2F1, has emerged as a central component of the cellular machinery that regulates a wide spectrum of genes involved in various functions. For E2Fs to perform with accuracy their transcriptional programs complex multi-level regulatory circuits govern their activity.

3.1. The E2F target genes

There is a great number of genes, implicated in cell cycle control, DNA licensing and synthesis, mitosis, DNA repair and apoptosis (Fig. 2, presented extensively in [32]), which contain the E2F responsive site in their

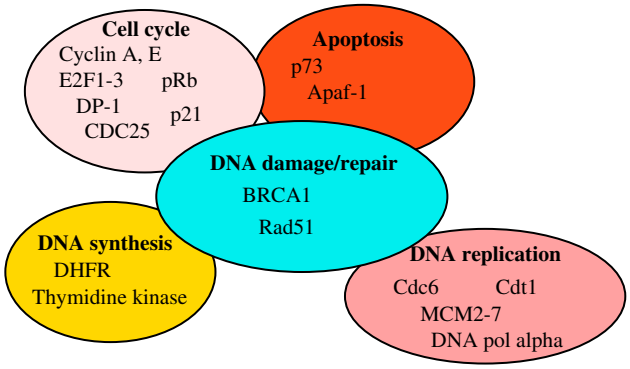

Fig. 2. The E2F target genes. The most important genes are presented and grouped according to function (also see Sections 3.1, 3.3, 3.4, and 3.5).

promoters [T/C]TT [C/G][G/C]C G[C/G] [33]. Interestingly, E2F1-3 can regulate their own transcription through the presence of E2F responsive sites in their promoters [34]. Although in the majority of the above-mentioned gene groups these sites have been shown to be functional, *in vitro* and *in vivo*, the actual gene expression pattern is not solely dependent on a particular E2F. Other key variables, such as the cell-cycle phase [35,9] (see Fig. 3) and the synergistic/antagonistic activity of other transcription factors also play a nodal role in determining the correct timing of gene expression. In this regard, E2F has been shown to interact with various transcription (*e.g.*, Sp1 [36,37]) and growth-regulatory factors [38] besides pocket proteins (see Section 3.2). Characteristically, it has been suggested that this former type of interaction may enable E2F to exert

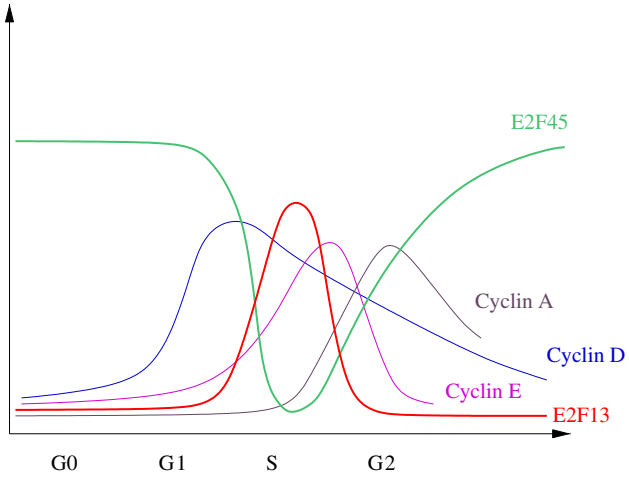

Fig. 3. The E2Fs and cell cycle progression. The orderly progression through the cell phases is orchestrated by the cyclins, cyclin-dependent kinases (CDKs), their inhibitors (CDKIs) and the members of the E2F family. Switching from "repressive" to "activating" E2Fs allows G_1 to S-phase transition. In this figure only the fluctuation of the cyclins and E2Fs, during the cell cycle, is presented (for details, also see Section 3.4).

transcriptional regulation on promoters that lack classical E2F responsive elements (reviewed in [38]).

Methylation of E2F responsive elements also appears to affect the expression of E2F target genes [39]. Surprisingly, some genes (*e.g.*, *DHFR*, *cdc2*, *E2F1*) are effectively silenced against E2F1-5 while others, (*e.g.*, *c-myc*, *c-myb*) remain responsive to E2F2 to 5, but not to E2F1.

3.2. The pocket-protein/E2F interaction

The E2F cell-cycle activity is mainly dependent on the phosphorylation state of the pocket proteins. The pocket protein family includes the retinoblastoma protein, pRb, which is the most prominent member [40], p107 [41] and p130 [42]. All of them share the ability to regulate cell cycle progression and arrest cells in G_1 when they are overexpressed [43,44]. There seems to be a certain degree of redundancy between p107 and p130. Mice deficient in pRb die as embryos, while mice deficient in either p107 or p130 develop normally [45,46]. This may explain, in part, the fact that p107 and p130 do not yet fully qualify as classical tumour suppressors (reviewed in [47]). Generally, when a pocket protein is hypophosphorylated it can physically associate with E2F, via the large A and B domains (pocket site) thus rendering it inactive. Moreover, the pocket protein/E2F-DP complex is guided to E2F binding sites where it can recruit histone deacetylases (HDACS) that suppress transcription by remodeling the nucleosome [48,49]. The first and most important pocket/E2F interaction detected was that between pRb and E2F1 [50–52], while the associations between the other E2Fs and pocket protein family members followed [53,54]. The active state of E2F is obtained when cyclin/CDKs phosphorylate the pocket protein relieving E2F from its inhibitory effect. Furthermore, free E2F has the ability to interact with histone acetyltransferases (HAT), such as CBP, which can act as co-activators [55].

In contrast to E2F-DP association, pocket protein binding shows considerable specificity for each E2F which also depends on the cell cycle phase (Figs. 1 and 3). The activating E2Fs (E2F1-3) bind selectively to pRb, while E2F5 binds p130 and E2F4 interacts with all pocket proteins [56,57].

3.3. Transcriptional regulation, post-translational modifications and degradation of E2F

Even though pocket protein interaction is the main mode of E2F regulation, transcriptional control, subcellular localisation (mentioned above) and post-translational modifications by various other pathways/proteins add several layers of complexity in E2F regulation.

The transcriptional regulation of E2Fs' levels is made possible through their respective promoters [58]. Initial

studies showed that the mouse E2F1 promoter contains an E2F-responsive site [34]. It is now widely accepted that the transcription of activating E2Fs is subjected to an E2F-dependent self-inhibitory loop [59–61] (Fig. 2). Activating E2F genes also contain Myc binding sites that are believed to enhance their transcription [60,62,63] at critical cell cycle points.

Post-transcriptionally, and in contrast with the other cyclin:CDK complexes [64], cyclin A:CDK2 phosphorylates E2F1 at serine 375 and increases its binding affinity for pRb [65]. Phosphorylation at serine 403 and threonine 433, within the activation domain, by the basal TF TFHII triggers E2F1 degradation [66]. Conversely, phosphorylation of E2F1, but not E2F2 or E2F3, by ATM/ATR induces E2F1 activity, by inhibiting its degradation [67], possibly through the action of the phosphoserine protein 14-3-3 tau [68]. Only free E2Fs are degraded by the ubiquitin system, since pocket protein binding stabilises them, increases their half-life [69] and at the same time blocks their synthesis, thus forming a negative feed-back loop.

The best studied E2F degradation pathway is that of E2F1. It depends upon ubiquitination by multi-part ubiquitin ligases. The E3 component of the ubiquitin conjugating complex that provides substrate specificity by binding selectively to E2F1 is the F-box protein $p45^{Skp2}$ [69]. The destruction of E2F1 and E2F3 can also take place in the nucleolar proteosome [70] via interaction with $p14^{ARF}$. This may provide an alternative regulatory circuit, given that E2F1 can induce $p14^{ARF}$ expression [71]. Newer evidence from *in vitro* studies show a possible role for ROC-cullin ligases [72], but their significance has not yet been fully clarified.

Acetylation is another type of post-translational modification that affects E2F activity. Recent studies have shown that acetylation of the activating E2Fs, but not the repressing ones, by the acetyltransferases p300 and CBP at lysine residues lying adjacent to the DNA binding domain increases their half-lives and DNA binding activity [73,74]. The effects of acetylation are reversible by the action of pRb-associated histone deacetylases [73,74].

3.4. E2F and cell cycle progression

The great number of cell replication-related genes that contain E2F binding sites underlines the vital role of E2F members in orchestrating cell cycle progression. A simplified view of the way these events take place is presented below (Fig. 3).

During G_0 and early G_1, E2F activity is mainly mediated by E2F4 and E2F5 which are bound preferentially to p130 and exert an inhibitory effect on the E2F-responsive genes. At the same time, the activating E2Fs are bound and inactivated by pRb. As the cell progresses to late G_1 phase, pRb and p130 are phosphorylated first by cyclin-D/CDK [75] and later by cyclin-E/CDK [76], thus, releasing the activating E2Fs. Simultaneously, p130 is targeted for ubiquitin-mediated degradation [77,78], its levels fall, and E2F4 and possibly E2F5 are shuttled from the nucleus to the cytoplasm [79]. It should be noted that the two *E2F3* gene products behave differently. E2F3b is constitutively expressed, in contrast to E2F3a whose concentration fluctuates during the cell cycle [61].

The end result of the above events is the activation of E2F1-3 and subsequent increased transcription of S-phase genes, including cyclin-E and cyclin-A (Figs. 2 and 3). Whether the cell will actually complete the cell cycle and divide or die via apoptosis depends, as we will see in the following sections, on the integrity of the replication process and the appropriate balance between E2F1 and growth factors.

3.5. E2F1 and apoptosis

Since E2F1 was first linked to apoptosis, approximately a decade ago [13,80], scientific research has focused on the underlying mechanisms of this intriguing relation. It seems that pro-apoptotic activity is an exclusive feature of E2F1 [14,81] and has been recently attributed to its marked-box domain [82]. Conventionally, the ability of a cellular factor to induce both proliferation and cell death would present a paradox. However, the reason behind this connection is that the cell, during its cell cycle course, should possess the ability to switch on a self-destruction program in the case of irreversible damage, especially during DNA synthesis. This task is better achieved if both mentioned cellular functions are co-ordinated by the same factor.

The pathways between E2F1 and apoptosis are multiple and interact in numerous ways (Fig. 4). The first E2F1-mediated apoptotic mechanism recognised was p53-dependent. It is based on E2F1's ability to induce the expression of the human tumour suppressor protein $p14^{ARF}$ [83,84] which in turn can bind directly to the p53 regulator, MDM2, preventing p53 degradation, increasing its stability and apoptotic activity. Recent studies, however, challenge the above mechanism by demonstrating, in both mouse and human cellular systems, that $p14^{ARF}$ is not strictly necessary for p53 activation and apoptosis by E2F1 [85–88]. Intriguingly, in normal cells $p14^{ARF}$ is constitutively repressed by E2F3 [89], thus, raising the threshold for apoptosis. In addition, current evidence indicates that E2F1 activates both p53 and Chk2 using the ATM pathway as a mediator [90,91]. Direct interaction between E2F1 and p53, possibly through the cyclin A binding site, has also been proposed to play some role in signalling apoptosis [92]. Finally, E2F1 can augment the apoptotic capacity of p53 by enhancing the transcription of pro-apoptotic p53 cofactors such as p53-ASPP1, ASPP2, JMY and TP53INP1 [93].

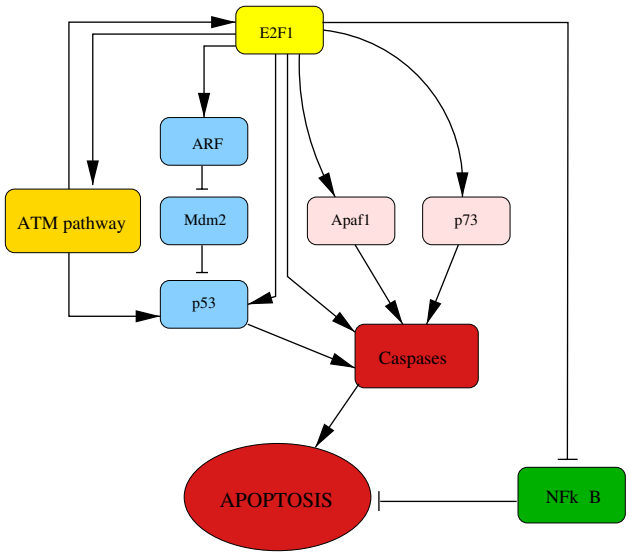

Fig. 4. E2F1 and apoptosis. The figure presents an outline of the three main mechanisms linking E2F1 with apoptosis: (i) the p53-dependent via p14ARF and ATM; (ii) the p53-independent via Apaf-1 and p73; (iii) the inhibition of anti-apoptotic signalling (for details see Section 3.5).

The apoptotic activity of E2F1 is also ensured by p53-independent mechanisms. The most important mediators are Apaf-1 [94,95], the p53 homologue p73 [96–98], caspase-8 and caspase-9 [99] and the pro-apoptotic members of the Bcl-2 family [100]. The two latter studies [99,100] clearly demonstrate that E2F1 is engaged in the apoptotic program of the cell. At the same time, E2F1 can also block anti-apoptotic signals by inhibiting NF-kappa B activation, possibly by inducing the degradation of the signalling complex that is formed at the TNF-receptor (TNFR) [101].

Conclusively, the integration of all these pathways provides a fail-safe network that guarantees a prompt apoptotic response. Nevertheless, under strongly favourable growth factor conditions, such as activation of the Akt protein kinase pathway [82], the apoptotic signals can be overwhelmed in favour of proliferation and cellular survival. It has been proposed that E2F1 may also, paradoxically, confer resistance to apoptotic stimuli, possibly by inhibiting pro-apoptotic target genes [102,103]. Still, the exact mechanism remains elusive.

3.6. E2F1 and DNA damage checkpoint control

The cell cycle is controlled by checkpoints that safeguard the accurate performance of DNA synthesis and cell division. DNA damage is a major impediment in cell cycle progression by setting in motion the DNA damage response (DDR) checkpoint [104]. The DDR comprises a complex network of proteins that trigger either growth arrest and subsequently DNA repair or apoptosis.

The ATM/ATR and Chk2 kinases are central molecules in this process. All of them share the ability to selectively phosphorylate E2F1. The ATM/ATR and Chk2 phosphorylation sites are located at Ser31 [67] and Ser364 [105] of the amino- and carboxy-termini of E2F1, respectively. Considering that E2F1 can use the ATM pathway to activate p53 and Chk2 [90] (see previous section, Fig. 4) it seems that during the DDR a positive feedback loop is formed between the two former proteins. ATM activation can also be interpreted as a by product of the DNA damage that is caused by inappropriate E2F1 action and the resulting unscheduled proliferation [106]. The connection between E2F1 and the DDR was hinted by early studies which demonstrated that ionising radiation (IR) increased the expression of E2F1 [107]. Subsequent studies revealed that DNA damage, due to IR or UV radiation, activates E2F1 in a manner analogous to p53 [108,109] and that MDM2 plays a major role in this process. This resemblance is based on the fact that both E2F1 and p53 share a strikingly similar MDM2 binding domain. Apart from irradiation, E2F1 is induced by other stress signals such as drugs and hypoxia. Notably, this response is independent of its transactivation function, pRb and p53 status [110].

Finally, as mentioned previously, E2F target specificity can be dictated by post-translational modifications. In the case of DNA damage, a recent study identified p73 as an important E2F1 mediator of the DDR and noted that acetylation is required for E2F1 to induce p73 transcriptional activation [111].

4. E2Fs in cancer

4.1. Lessons from cell lines and mice

The cell cycle cannot progress without the activating E2Fs, a fact that is readily evident in *E2F1*$^{-/-}$ and *E2F3*$^{-/-}$ mouse embryo fibroblasts (MEFs) which show impaired cell cycle entry and delayed onset of DNA synthesis, respectively [112,113]. These results suggest that despite their functional redundancy, E2F1 and E2F3 possess unique roles during cell division. Notably, this difference is extended to the developmental process with *E2F1*$^{-/-}$ mice manifesting minor developmental defects compared to *E2F3*$^{-/-}$ mice which demonstrate a high frequency of neonatal lethality [112,114]. Thus, one would anticipate high levels of free activating E2Fs (E2F1-3) to convey an increased oncogenic capacity. Indeed, cells transfected with *E2F1* can form colonies in soft agar, induce tumour formation in nude mice and, in cooperation with activated *ras*, transform rat embryo fibroblasts (REFs) [115]. E2F1 can also overcome transforming growth factor-beta (TGF-beta) mediated suppression [116] and increase the susceptibility to skin tumours [117,118] in p53-deficient (null or heterozygous) mice. Moreover, transgenic K5-E2F1 expression (*E2F1*

gene under the control of a keratin 5 (*K5*) promoter) induces spontaneous tumours of epithelial basal cell origin [119], whereas forced expression of E2F1 in the liver results in hepatocellular adenomas and large cell dysplasias [120]. Similarly, E2F2 can lead thymic epithelial cells to unscheduled proliferation and thymic tumour development, as demonstrated in a transgenic mouse model [121].

On the other hand, E2F1 possesses the ability to induce p53-dependent and p53-independent apoptosis, a function which implies a putative oncosuppressor activity. This hypothesis was confirmed by Yamasaki *et al.*, who demonstrated that mice lacking E2F1 developed a broad spectrum of tumours [122], a finding which was later confirmed in other transgenic systems [123]. Intriguingly, it seems that the oncosuppressor activity is not limited to E2F1, but is expanded to other E2F members as well. Particularly, E2F3 has been shown to display such attributes in certain transgenic mice settings [124] possibly by contributing to the apoptotic process [125,124]. The latter characteristic alters the prevailing view that E2F1 is the only E2F member to promote apoptosis. In addition, $E2F2^{-/-}$ mice on an $E2F1^{+/-}$ background show increased incidence of tumourigenesis, suggesting that E2F2 may also bear tumour suppressor properties [121]. This has also been suggested in previous mouse studies [126,127].

However, things in nature are rarely so simple. For example, loss of *E2F1* is not always associated with enhanced tumour development. In Rb heterozygous mice, loss of *E2F1* impairs the development of pituitary and thyroid tumours, which represent hallmarks of mice carrying a germ-line Rb mutation. In the same mice, tumour incidence increases within certain tissues and decreases in others [128]. Comparable findings have been observed in mice that are $E2F3^{-/-}/Rb^{+/-}$, although in this setting loss of *E2F3* alters the tumour spectrum by suppressing pituitary tumour development and promoting the growth of medullary thyroid carcinomas [124]. Hence, the results in compound *Rb*/*E2F* mutant mouse models can only partially explain how the growth suppressive properties of pRb relates to its role in the inhibition of the activating E2Fs. Furthermore, the lack of a complete rescue implies that additional E2F members might contribute to the tumour phenotype. The finding that *E2F4* loss greatly restrains tumour formation in $Rb^{+/-}$ mice [129] further supports this notion.

A definite conclusion that can be drawn from the above presented reports is that, regardless of the underlying mechanism, the E2F proteins and particularly the activating members display both tumour promoting and suppressing capabilities in mice. It is also apparent that this bimodal action is highly context dependent, greatly influenced by the presence or absence of pRb and the levels and/or activities of the remaining E2F species.

4.2. E2Fs and human malignancies

Compared to the many *in vitro* studies and the significant number of investigations on simple *E2F* or compound *RB*/*E2F* transgenic models, there are few reports dealing with this nodal group of transcription factors in human malignancies. In stark contrast, the pRb pathway, which mainly regulates E2F activity, and its downstream effector p53 have been extensively studied in human tumours [130].

Prompted by E2F's bimodal behaviour in various *in vitro* cellular systems and *in vivo* animal models, our group has designed a series of studies in order to investigate their role in common human cancers. Initially, we focused on the founding member E2F1 and examined its status and relationship with the kinetic parameters of proliferation and apoptosis in a panel of non-small cell lung carcinomas (NSCLCs) [131,132]. We observed: (i) that E2F1 protein levels were significantly higher in the cancerous area compared to the adjacent normal tissue [131] and (ii) a high degree of phospho-pRb and E2F1 co-localisation suggesting that overexpressed E2F1 corresponded to the free and active form [132]. Moreover, and in accordance with a recent study [133], elevated E2F1 levels were associated with aberrant pRb status, further supporting the notion that E2F1 was unleashed from the inhibitory effect of pRb [131]. The analysis of tumour kinetics revealed the most important correlation, showing that increased E2F1 was positively associated with the tumour growth index. The main determinant of this correlation was the parallel increase between E2F1 and proliferation, whereas apoptosis was not influenced. This latter result implied that the link between E2F1 and apoptosis, in this type of cancer, is disrupted. Indeed, in our series, high E2F1 expression was frequently associated with deregulation of the p53-MDM2 regulatory loop [131], a common phenomenon in NSCLCs [134,135]. An additional finding was that patients with increased E2F1 positivity had a poorer outcome, thus, strengthening the concept that in the NSCLC environment E2F1 can act as an oncogene. A similar role for E2F1 has been proposed by Eymin *et al.* in a series of neuroendocrine lung tumours [136]. Notably, in a recent study, adenovirus-mediated E2F1 gene transfer inhibited the growth of NSCLC cell lines and tumours implanted in SCID mice by inducing apoptosis [137]. A putative explanation for this discrepancy is that "forced" expression of E2F1 does not fully recapitulate the endogenous status and behaviour of E2F1 within a specific cellular context. Thus, exogenous administration of E2F1 probably overcomes the higher threshold required for apoptosis, whereas induction of proliferation is achieved by lower amounts of the protein. Similar results have been observed in colon cancer cell lines by E2F1 adenoviral transfer [138,139].

In breast, thyroid [140,141] and pancreatic [142] cancer, the role of E2F1 seems to follow the scenario which applies for lung cancer. In a series of breast carcinomas our group observed that, within a setting of abnormal expression of the $p16^{INK4a}$–$p27^{Kip1}$–pRb network [143,144] and defective p53 status, increased expression of the free and active E2F1 was positively related with tumour growth [144]. In the same context, the study of Zhang *et al*. proposes E2F1 expression as a proliferative marker of breast neoplasia [145]. In another report, E2F1 expression was shown to predict a worse outcome in lymph node positive breast cancer patients treated with adjuvant chemotherapy, suggesting that its expression may be used as a biological marker of treatment response [146]. Besides E2F1, other members of the family which appear to play a role in breast cancer are E2F4 and E2F5. Bearing in mind that the chromosomal region 16q22 which includes E2F4 (Fig. 1) is frequently deleted in breast cancer [147,148], one would expect for E2F4 to behave as an oncosuppressor in breast cancer. Nevertheless, a comprehensive study by Rakha *et al*. revealed that E2F4 nuclear immunoreactivity was associated with breast cancer progression and adverse prognosis, suggesting that it is not the gene targeted by 16q22.1 loss in this type of cancer [149]. Amplification of the *E2F5* gene, followed by increased transcription, has been reported in breast cancer [150]. Interestingly, *E2F5* is mapped to 8q21, a region frequently amplified in breast cancer, which also harbors the *c-myc* and *c-mos* oncogenes. This finding indicates that *E2F5* alone or in cooperation with other regional oncogenes may have a role in breast cancer development. The significance of E2F4 and E2F5 in this type of cancer may also lie in their ability to mediate the transcription of estrogen receptor alpha [151].

The study of transitional cell bladder carcinomas (TCCs) presents controversial findings. Rabbani *et al.* [152] were the first to investigate E2F1 in TCCs and suggested a tumour suppressive role, based on the fact that low E2F1 correlated with a worse prognosis. However, in our series, we observed a positive correlation between E2F1 and proliferation, but not with apoptosis, which implies a growth promoting rather than a growth suppressive effect [144]. We believe that the discrepancy between these reports lies in the type of TCC examined. Our study was conducted in superficial low-grade TCCs while, the material examined by Rabbani, mainly comprised invasive bladder tumours which appear to possess distinct molecular and kinetic characteristics compared to superficial TCC [153]. Another E2F family member, E2F3, is probably an important contributor to bladder cancer progression since the *E2F3* gene was frequently found to be amplified [154–156] and its overexpression was associated with tumour proliferation and invasiveness [155].

Although E2F1 overexpression is a relatively common event in colon cancer [144,157–159], it appears that its expression opposes tumour growth pointing towards an oncosuppressor role. Examining the relationship between E2F1 and tumour kinetics in serial sections, we noticed a distinct reverse topographical distribution of E2F1 and Ki-67. In addition, the areas with E2F1 immunoreactivity demonstrated higher apoptotic index values. Therefore, we suggested that within the context of colon cancer, E2F1 behaves as an oncosuppressor by inducing the apoptotic pathway [144]. The manner in which it appears to act in large B-cell lymphomas is analogous [160]. Moreover, we hypothesised that apoptosis may be mediated by a p53-independent pathway due to the high frequency of p53 alterations detected [144]. The association between E2F1 and apoptosis may also explain the paradoxical progressive increase in pRb expression during the various steps of colon carcinogenesis [139], which possibly represents a "protective" barrier formed against the deleterious effects of E2F1. Finally, an issue that merits further clarification concerning E2F1's role in gastrointestinal tumours is the significance of its gene amplification, which has been reported in several cases of gastric and colorectal carcinomas [158,161]. In contrast to the above, E2F4 is frequently overexpressed in colon cancer but seems to exert a protective effect against apoptosis [162]. Moreover, the *E2F4* gene possesses a serine repeat trinucleotide, $(AGC)_n$, which is frequently mutated in various tumours including replication error positive gastrointestinal tumours [163–165]. It remains unclear whether these genetic alterations affect E2F4 function or simply reflect secondary replication errors. It is noteworthy that, with the exception of the trinucleotide repeat region in *E2F4*, the *E2F* family is generally not a common target for mutations in cancer as we [131] and others [165] have noticed (also reviewed in [166]).

The analysis of prostatic carcinomas revealed several interesting findings. E2F1 was characteristically absent in the cancerous areas, whereas it was expressed in the normal prostatic parenchyma [144]. This result implies either oncosuppressor activity or that the E2F activity required for proliferation may be provided by some other members. Indeed, the latter appears to be valid since E2F2 was expressed in both prostatic cancer samples and cell lines [144]. In accordance with this hypothesis, E2F3 is also overexpressed in prostatic carcinomas and associated with poorer survival. The effect of E2F3 is possibly mediated through the expression of EZH2, which has been recently implicated in the development of human prostate cancer [167]. A parameter that may play a role in the absence of E2F1 expression is the interplay with the androgens, a well established force driving prostate tumour growth [168]. It has been shown that high synthetic androgen levels may increase pRb activity, thus hindering E2F1 action [169], while androgen withdrawal leads to increased E2F1 expression [170]. This interaction may provide valuable clues towards understanding E2F1 behaviour in prostate cancer.

4.3. E2Fs and genomic instability

Genomic instability is a prime requisite that allows the cancer cell to thrive and adapt. The transition to the degenerate malignant phenotype is realised via the accumulation of DNA alterations that deregulate the normal cellular settings [171]. In that sense, the disruption of the mechanisms that protect the cell from DNA defects appears to be the earliest and most significant step towards cancer [104,106,172]. As stated in the previous sections, the coordinated action of the E2Fs, and especially the relative balance between activating and repressing activities, guarantees to a significant degree an orderly cell cycle progression without affecting the integrity of the genome. The type of defects that are prevented by the accurate execution of the S and mitotic phases are DNA re-/under-replication and chromosomal instability (CIN), respectively.

Re-replication poses a serious threat to DNA integrity and is disallowed by the replication licensing mechanism which ensures one DNA replication per cell cycle. The activating E2F members play a vital role in this process since they target the nodal factors of the replication licensing apparatus [173–175,132]. Based on this fact, a link between E2F overexpression, deregulation of the replication licensing factors and genomic instability may exist in cancer. In accordance with this notion, we observed in our database of lung cancer patients a significant association between increased E2F1 and hCdt1 levels, a finding which was further supported by their functional relationship [175,132]. Moreover, patients with overexpression of the replication licensing factors hCdc6 and/or hCdt1 were associated with genomic instability when p53 was mutated [132]. These findings support the concept that deregulation of the licensing machinery elicits a DDR, which must be overridden for the cancer cell to progress. Another way by which overexpression of the activating E2Fs can lead to genetic instability is shortening the G_1-phase and, eventually, directing the cell illegitimately into the S-phase [12,176] (also, reviewed in [177]). As we have recently shown, this can cause replication stress, DDR and genomic instability, even in the earliest stages of cancer development [172]. Finally, there are indications that deregulated expression of E2Fs can result in CIN. To this end, in a recent study, loss of *E2F3* led to disruption of the centrosome duplication cycle, centrosome amplification, mitotic spindle defects and aneuploidy [178].

5. Conclusions and perspectives

Understanding the function of E2Fs is a challenging task that will influence our current perspective of cancer. It is true that E2Fs, and primarily E2F1, seem to defy a conventional classification into ''oncogenes'' and ''tumour suppressors''. The orchestrated action of all E2F family members can only be comprehended in a specific cellular context. The gradual accumulation of knowledge from human malignancies is likely to allow researchers to draw meaningful conclusions in the quest for new prognostic markers and, possibly, promote the development of novel anti-cancer agents based on E2F biology (reviewed in [179]).

Conflict of interest statement

None declared.

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