Atlas of Genetics and Cytogenetics in Oncology and Haematology

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# Gene Section

## Review

# E2F4 (E2F transcription factor 4, p107/p130-binding)

**Marie-Christine Paquin, Nathalie Rivard**

CIHR Team on Digestive Epithelium, Departement d'Anatomie et Biologie Cellulaire, Faculte de Medecine et des Sciences de la Sante, Universite de Sherbrooke, Sherbrooke, QC, Canada (MCP, NR)

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## Identity

**Other names:** E2F-4

**HGNC (Hugo):** E2F4

**Location:** 16q22.1

## DNA/RNA

### Description

E2F4 gene spans 6753 base pairs.

### Transcription

E2F4 gene produces 1 transcript of 2100 bp that counts 10 exons.

| Exon/Intron | Start | End | Length (bp) |
|---|---|---|---|
| 5' upstream | | | |
| Exon 1 | 67,226,068 | 67,226,265 | 198 |
| Intron 1-2 | 67,226,266 | 67,226,663 | 398 |
| Exon 2 | 67,226,664 | 67,226,773 | 110 |
| Intron 2-3 | 67,226,774 | 67,226,911 | 138 |
| Exon 3 | 67,226,912 | 67,227,073 | 162 |
| Intron 3-4 | 67,227,074 | 67,227,374 | 301 |
| Exon 4 | 67,227,375 | 67,227,418 | 44 |
| Intron 4-5 | 67,227,419 | 67,228,300 | 882 |
| Exon 5 | 67,228,301 | 67,228,362 | 62 |
| Intron 5-6 | 67,228,363 | 67,228,588 | 226 |
| Exon 6 | 67,228,589 | 67,228,883 | 295 |
| Intron 6-7 | 67,228,884 | 67,229,684 | 801 |
| Exon 7 | 67,229,685 | 67,229,909 | 225 |
| Intron 7-8 | 67,229,910 | 67,231,501 | 1592 |
| Exon 8 | 67,231,502 | 67,231,549 | 48 |
| Intron 8-9 | 67,231,550 | 67,231,769 | 220 |
| Exon 9 | 67,231,770 | 67,231,814 | 45 |
| Intron 9-10 | 67,231,815 | 67,231,910 | 96 |
| Exon 10 | 67,231,911 | 67,232,821 | 911 |
| 3' downstream | | | |

Table adapted from Ensembl.

### Pseudogene

E2F4P1 (E2F Transcription Factor 4, p107/p130-binding pseudogene 1).
Location: 6p21.2 (39521587-39522719).

## Protein

### Description

The E2F4 coding sequence yields a 413-amino acid protein predicting a molecular weight of 44 kDa. However, E2F4 migrates as an heterogeneous set of bands between 57-64 kDa which are associated with extensive phosphorylations (Beijersbergen et al., 1994; Ginsberg et al., 1994; Vairo et al., 1995; Gaubatz et al., 2001; Popov et al., 2005; Araki et al., 2008; Scime et al., 2008; Van Hoof et al., 2009; Litovchick et al., 2011).

Unlike E2F1, E2F2 and E2F3, which exhibit a cyclin A binding domain at their N-terminus, E2F4 has a truncated N-terminus and therefore does not harbor this domain (Beijersbergen et al., 1994; Sardet et al., 1995).

The full E2F transcriptional activity requires its heterodimerization with a DP partner, although E2F transcription factors have been reported to bind DNA as homodimers (Bandara et al., 1993; Helin et al., 1993b; Huber et al., 1993; Krek et al., 1993). The DNA-binding domain of E2F4 was originally viewed as a helix-loop-helix DNA-binding motif (Kaelin et al., 1992; Cress et al., 1993).

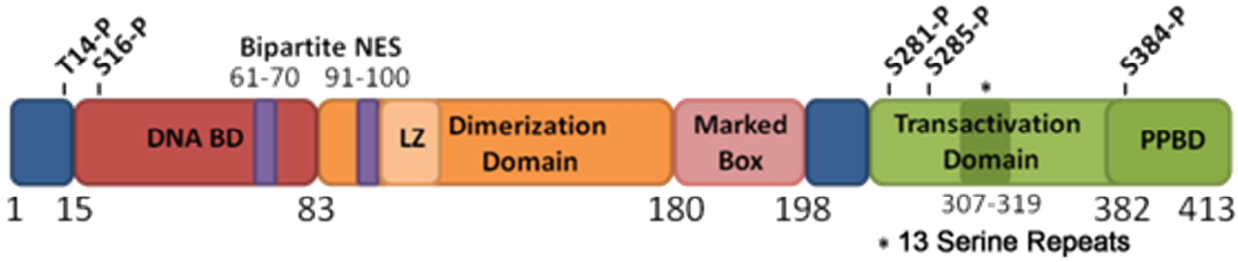
However, the crystal structure of E2F4-DP2 dimer by Zheng et al. rather reveals a structure related to the winged-helix DNA-binding motif. E2F4 and its DP partner bind to the E2F-consensus DNA sequence TTTC/GC/GCGC/G (Nevins, 1992; Slansky et al., 1993) by means of a conserved Arg-Arg-Xxx-Tyr-Asp sequence (Zheng et al., 1999). Binding specificity of

E2F transcription factors to different target gene promoters is affected by the DNA sequence itself, E2F transcription factors, DP partners as well as other factors (Karlseder et al., 1996; Lin et al., 1996; Shin et al., 1996; Wells et al., 1997; Le Cam et al., 1999; Chen et al., 2002; Schlisio et al., 2002; Araki et al., 2003; Giangrande et al., 2003; Giangrande et al., 2004; Zhu et al., 2004).

The transactivation domain of E2F transcription factors, including E2F4, mediates target gene transcription through two distinct mechanisms: 1- by recruiting general transcription machinery such as TBP/TFIID, TFIIA and TFIIH which promote RNA pol II pre-initiation complex (PIC) assembly (Hagemeier et al., 1993; Emili and Ingles, 1995; Pearson and Greenblatt, 1997; Ross et al., 1999; Vandel and Kouzarides., 1999) and 2- by relaxing chromatin structure at promoters by interacting with histone acetyltransferases (HAT) such as Tip60 (Taubert et al., 2004), p300/CBP (Trouche and Kouzarides, 1996; Trouche et al., 1996; Martinez-Balbas et al., 2000; Marzio et al., 2000) and PCAF/GCN5 (Martinez-Balbas et al., 2000; Marzio et al., 2000; Lang et al., 2001). The transactivation domain also includes a pocket protein interacting domain. E2F4 interacts primarily with p130/RBL2, p107/RBL1 and to a lesser extent with Rb/RB1 (Beijersbergen et al., 1994; Ginsberg et al., 1994; Ikeda et al., 1996; Moberg et al., 1996; Li et al., 1997). Pocket proteins modulate E2F transcription

factor activity via two different mechanisms: 1- by preventing general transcription machinery and chromatin-remodeling protein recruitment (Helin et al., 1992; Flemington et al., 1993; Hagemeier et al., 1993; Helin et al., 1993a; Pearson and Greenblatt., 1997) and 2- by actively repressing gene transcription (Harbour and Dean, 2000; Singh et al., 2010). In fact, pocket proteins have been shown to recruit histone deacetylase enzymes (HDACs) (Brehm et al., 1998; Luo et al., 1998; Dahiya et al., 2000), the histone methyltransferase SUV39H1 (Nielsen et al., 2001; Vandel et al., 2001), SWI/SNF family members (BRG1, Brm) (Dunaief et al., 1994; Singh et al., 1995; Strobeck et al., 2000; Zhang et al., 2000; Iakova et al., 2003), the Sin3B repressor complex (via RBP1 and SAP30) (David et al., 2008; Grandinetti and David., 2008) and the ErbB3 binding protein Ebp1 (Zhang et al., 2003), all of which contribute to chromatin compaction and thus, to transcriptional repression (Kouzarides, 2007).

During quiescence, E2F transcription factors are sequestered by hypophosphorylated forms of pocket proteins (pRb, p130 and p107) which prevent the activation of their target genes. Upon G1 progression, cyclin-dependent kinases (cdk)-cyclin complexes are activated and phosphorylate pocket proteins leading to the release of E2F transcription factors. Indeed, activation of cdk4 and cdk6, in association with cyclin D, leads to partial inactivation of Rb-like proteins. Further phosphorylation by cyclin E/cdk2 complex is required for total pocket protein inactivation and full release of E2F transcription factors.




Adapted from Sardet et al., 1995; Magae et al., 1996; Zheng et al., 1999; Scime et al., 2008.

**DNA BD:** Originally viewed as a basic helix-loop-helix DNA-binding motif (Kaelin et al., 1992; Cress et al., 1993), however the crystal structure of E2F-4/DP2 dimer binding to DNA revealed a winged-helix DNA-binding motif (Zheng et al., 1999).

**Dimerization Domain:** Dimerization with DP proteins is required for proper E2F DNA binding and transactivation capacity. The **Leuzine Zipper** (100-128) and the **Marked Box** both contribute to DP dimerization (Kaelin et al., 1992; Chen et al., 2009). E2F transcription factors can bind DNA as homodimers although to a much lesser extent than heterodimers (Huber et al., 1993).

**Transactivation Domain:** The transactivation domain mediates interaction with transcriptional machinery, co-activators and chromatin-remodeling proteins (Emili and Ingles, 1995; Trouche and Kouzarides, 1996; Pearson and Greenblatt, 1997; McMahon et al., 1998; Ross et al., 1999; Vandel and Kouzarides, 1999; Martinez-Balbas et al., 2000; Marzio et al., 2000; Lang et al., 2001; Louie et al., 2004; Taubert et al., 2004) and includes the **Pocket Protein Binding Domain**. E2F4 interacts with p130/RBL2, p107/RBL1 and to a lesser extent with pRb/RB1 (Beijersbergen et al., 1994; Ginsberg et al., 1994; Ikeda et al., 1996; Moberg et al., 1996; Li et al., 1997). Association of E2F4 with Rb protein family members (particularly p130/RBL2) prevents activation of E2F target genes and triggers their repression by recruiting transcriptional repression machinery (Zhang and Dean, 2001; Frolov and Dyson, 2004; Litovchick et al., 2007). E2F4 is the only E2F possessing a stretch of 13 consecutive serine residues in its transactivation domain (Sardet et al., 1995).

Numerous publications infer that E2F4 is a phosphorylated protein (Beijersbergen et al., 1994; Ginsberg et al., 1994; Vairo et al., 1995; Gaubatz et al., 2001; Popov et al., 2005; Scime et al., 2008) although only a few have identified specific phosphorylation sites: **T14** and **S16** are phosphorylated residues (Van Hoof et al., 2009); **S281** and **S285** are phosphorylated by IKKα and IKKβ and enhance E2F4 nuclear localization and DNA-binding of the E2F4/p130 complex in human primary fibroblasts (Araki et al., 2008); **S384** is phosphorylated in the DREAM or MMB complexes (Litovchick et al., 2011).

Thereafter, genes required for DNA synthesis and cell cycle progression are induced, allowing cells to enter S-phase and pursue their cell cycle (Cobrinik, 2005; Malumbres and Barbacid, 2009).
In addition to pocket protein-mediated regulation, E2F4 is also controlled by other mechanisms such as phosphorylation, antisenses (Yochum et al., 2007), reactive oxygen species (Kim and Lee, 2010), cofactors and mainly by its subcellular localization. Indeed, E2F4 protein levels are not significantly modulated during cell cycle progression; however, its nuclear localization is tightly regulated (Lindeman et al., 1997; Verona et al., 1997; Deschenes et al., 2004) (see below). Furthermore, many studies have reported E2F4 phosphorylation but only a few have associated these phosphorylation events with a specific function (Beijersbergen et al., 1994; Ginsberg et al., 1994; Vairo et al., 1995; Gaubatz et al., 2001; Popov et al., 2005; Araki et al., 2008; Scime et al., 2008; Van Hoof et al., 2009; Litovchick et al., 2011). For example, Araki et al., 2008 showed that E2F4 phosphorylation by IKKα and/or IKKβ leads to increased binding of the E2F4/p130 complex to DNA in TIG-3 human primary fibroblasts.
Finally, Balciunaite et al., 2005 suggested that in early $G_1$, both p107 and p130 are absent of certain repressed E2F4 target promoters, suggesting that another repression mechanism must be implicated. Moreover, Rayman et al., 2002 reported that pocket proteins are not required for mSin3B recruitment to certain E2F-regulated promoters reinforcing the notion of a pocket protein-independent repression mechanism. Indeed, E2F4 can also recruit Host cell factor-1 (HCF-1), an important cell cycle regulator, which brings Sin3/HDAC complexes to E2F target gene promoters, repressing their transcription independently of pocket proteins (Tyagi et al., 2007).

## Expression

In adult mouse tissues, Rempel et al. proposed that E2F4 is particularly abundant in hematopoietic tissues (i.e. thymus, spleen and bone marrow) and in the gut, in comparison to the heart, kidney, liver, brain and muscle (Rempel et al., 2000). However, other publications reported an ubiquitous expression of E2F4 (Gill and Hamel, 2000; D'Souza et al., 2001). Furthermore, it was suggested that E2F4 accounts for the vast majority of E2F cellular activity (Moberg et al., 1996; Puri et al., 1997). Of note, E2F4 expression changes according to cellular status. During external epithelium formation, E2F4 transcripts are detectable in 11,5-day post coitum (dpc) embryos in all cells of the ectoderm. In 13,5-dpc embryos, the proliferating undifferentiated epithelial cells show strong E2F4 expression. At 18,5-dpc, proliferating basal cell layers of the primitive epidermis express abundant E2F4 transcripts whereas the suprabasal cell layers display negligible E2F4 signals (Dagnino et al., 1997b). During intestinal morphogenesis, E2F4 expression is high in the intervillus epithelium and almost absent in the non proliferating villus tips and in the underlying mesenchyme (Dagnino et al., 1997b). E2F4 transcripts are widespread in the central and peripheral developing nervous systems. In the developing brain, E2F4 is ubiquitously expressed with the highest levels detected in ventricular and subventricular zones (Dagnino et al., 1997a; Ruzhynsky et al., 2007).

## Localisation

E2F4 exhibits a CRM1-dependent bipartite nuclear export signal (NES) which mediates its export to the cytoplasm. The two elements of E2F4 NES are referred to as NES1 (residues 61 to 70) and NES2 (residues 91 to 100) (Gaubatz et al., 2001). Therefore, E2F4 relies on other proteins to reach the nucleus, such as DP2 (Magae et al., 1996; Verona et al., 1997; Puri et al., 1998) or pocket proteins (Verona et al., 1997; Gaubatz et al., 2001; Apostolova et al., 2002; Rayman et al., 2002). Studies have shown that nuclear E2F4, triggered by the addition of an ectopic NLS or co-expression with DP2, is transcriptionally active and can induce DNA synthesis (Lindeman et al., 1997; Muller et al., 1997; Verona et al., 1997; Puri et al., 1998; Gill and Hamel, 2000). Of note, Apostolova et al. suggested that E2F4 may also travel to the nucleus on its own (Apostolova et al., 2002).
A number of studies have highlighted the importance of regulating the subcellular localization of E2F4 (Magae et al., 1996; Lindeman et al., 1997; Muller et al., 1997; Verona et al., 1997; Puri et al., 1997; Puri et al., 1998; Gill and Hamel, 2000; Deschenes et al., 2004). In immortalized fibroblasts and certain cancer cells, E2F4 is expressed in the nucleus of quiescent cells and as cells progress through G1 and enter the S phase, E2F4 translocates to the cytoplasm (Lindeman et al., 1997; Muller et al., 1997; Verona et al., 1997). In addition, overexpression of E2F4 in certain asynchronously growing cancer cells or fibroblasts revealed that E2F4 is primarily expressed in the cytoplasm (Magae et al., 1996; Lindeman et al., 1997; Muller et al., 1997; Verona et al., 1997). By contrast, endogenous E2F4 is found in the nucleus of proliferating basal epidermal cells (Paramio et al., 2000), proliferating intestinal crypt cells (Deschenes et al., 2004) and proliferating cardiomyocytes (van Amerongen et al., 2009). Likewise, overexpression of E2F4 in mice epidermis under the K5 promoter leads to E2F4 expression in the nucleus of cycling keratinocytes in the basal cell layer and the hair follicle resulting in hyperplasia and increased tumor formation in a mouse skin model of multistage carcinogenesis (Wang et al., 2000). Moreover, endogenous E2F4 is observed in the nucleus of many differentiated cells including ciliated epithelial cells (Danielian et al., 2007), myotubes (Puri et al., 1997; Puri et al., 1998) and neurons (Persengiev et al., 1999). Overall, these results suggest that E2F4 can act as either an activator or an inhibitor of transcription,

proliferation and differentiation. Therefore, E2F4 localization control must be tightly regulated in a timely and restricted manner.

## Function

The main characterized function of E2F transcription factors is the regulation of the cell cycle. E2F factors induce a number of genes required for DNA synthesis, including dihydrofolate reductase, thymidine kinase, DNA polymerase α, and for cell cycle progression, including cyclin A, cyclin E, c-myc, E2F and cdc2, to name a few (Stevens and La Thangue, 2003; Tsantoulis and Gorgoulis, 2005). In quiescent fibroblasts and certain cancer cells, E2F4 (mainly in complex with p130) binds to DNA and contributes to active repression of E2F target genes preventing cell cycle progression (DeGregori, 2002; Trimarchi and Lees, 2002; Cam et al., 2004). Upon G1 phase progression, E2F4 translocates to the cytoplasm while E2F1-3a transactivates genes required for S phase entry (Takahashi et al., 2000; Rayman et al., 2002; Trimarchi and Lees, 2002). However, no detectable defect either in cell cycle regulation or target gene expression was identified in $E2F4^{-/-}$ mouse embryonic cells, suggesting its compensation by other E2F members (Humbert et al., 2000; Rempel et al., 2000; Landsberg et al., 2003). Accordingly, dual loss of E2F4 and E2F5 impairs pocket protein-mediated cell cycle exit (Gaubatz et al., 2000).

Interestingly, loss of p107 and p130 triggers a massive E2F4 relocalization to the cytoplasm accompanied by a hyperacetylation of nucleosomes proximal to E2F binding sites, producing an important de-repression of E2F target genes (Rayman et al., 2002). Indeed, during quiescence, E2F4/p130 or E2F4/p107 complexes are associated with E2F-responsive genes and repress their transcription (Takahashi et al., 2000; Wells et al., 2000; Rayman et al., 2002). Recent data also implicate E2F4 as part of a multiprotein complex referred to as the DREAM complex (DP, RB-like, E2F4 and MuvB). In fact, the DREAM complex binds to the promoters of more than 800 cell cycle-regulated genes during quiescence and favors their repression (Litovchick et al., 2007; Schmit et al., 2007). This E2F4 repressing effect is also observed during cell cycle arrest associated with aging and differentiation.

For example, in aging mice, reduced proliferation of hepatocytes is accompanied by the association of Brm1/C/EBPα/E2F4/Rb repressive complex to E2F target genes (Iakova et al., 2003). In keratinocytes, E2F4 in complex with p130 recruits HDAC1 and represses Cdc25A, correlating with cell cycle arrest (Iavarone and Massague, 1999).

In keeping with the above concept, (Grandinetti and David, 2008) proposed the following model. Upon entry into quiescence, repression of E2F responsive genes responsible for cell cycle progression is initiated by the recruitment of E2F4/5 bound to a Rb-like protein (pRb, p130, p107) to target promoters. Sin3B recruitment to the pocket protein is then brought by Retinoblastoma Binding protein 1 (RBP1) and Sin3 Associated Protein 30 (SAP30) adaptor proteins. Thereafter, Sin3B recruits HDACs, which desacetylate histones, and RBP2, a histone demethylase responsible for demethylation of histones on lysine 4. All of these events promote nucleosome assembly rendering chromatin less permissive to transcription. Upon permanent cell cycle withdrawal (terminal differentiation or senescence), further recruitment of SUV39H1 enables methylation of histones on lysine 9 creating docking sites for HP1 protein and thus driving heterochromatinization and stable repression of E2F target genes (Narita et al., 2003; Grandinetti and David, 2008).

Although E2F4 has been particularly described as a repressor of both transcription and cell cycle progression (Vairo et al., 1995; Muller et al., 1997; Rayman et al., 2002), several studies have reported other roles such as 1- its binding to E2F-responsive elements as a pocket protein-free E2F during S phase, 2- its capacity to induce E2F target genes and 3- its implication in proliferation. Hence, these studies suggest that E2F4 can also act as a transcriptional activator (Verona et al., 1997; Wells et al., 1997; Ross et al., 1999; Lang et al., 2001; Garneau et al., 2009; van Amerongen et al., 2009). For example, studies carried out by Lo et al., 2011 demonstrated that the majority of E2F4 binding sites are located proximal to transcription start sites. There, E2F4 has been shown to stabilize TFIID/TFIIA complex thereby preventing Rb repressor effect and promoting PIC assembly (Ross et al., 1999). E2F4 can recruit the potent acetyltransferase GCN5 and the cofactor TRRAP which promote E2F4 transcriptional activity (Lang et al., 2001).

Host cell factor-1 also interacts with E2F4 and plays both co-activator (Knez et al., 2006) or co-repressor (Tyagi et al., 2007) roles in the regulation of E2F4-controlled promoters. Accordingly, forced expression of nuclear E2F4 promotes S-phase entry into cardiomyocytes (Ebelt et al., 2005; van Amerongen et al., 2009).

Moreover, nuclear E2F4 expression is associated with proliferation of rapid renewing tissues such as bone marrow (Kinross et al., 2006; Zhang et al., 2010), digestive tract (Rempel et al., 2000; Garneau et al., 2009) and skin (Wang et al., 2000; Wang et al., 2001).

Many in vivo and in vitro studies have led to the identification of numerous roles of E2F4 in different cellular processes such as nervous system development, intestinal homeostasis, bone development, myogenesis, adipogenesis and erythropoiesis, to name a few. E2F4 gene deletion in mice leads to important neonatal lethality due to chronic rhinitis and increased susceptibility to opportunistic infections (Humbert et al., 2000). Many factors contribute to the observed neonatal lethality in these mice. First, ciliated cells are absent from the entire airway epithelium and are replaced by mucin-secreting cells, creating a mucus

overflow in the nasal cavities allowing microbial colonization (Danielian et al., 2007). Secondly, sonic hedgehog (Shh) signaling is dysregulated which impairs eye patterning, self-renewal capacity of neural progenitor cells and ventral telencephalic structure formation during brain development (Ruzhynsky et al., 2007; Ruzhynsky et al., 2009; Swiss and Casaccia, 2010). Studies also highlighted E2F4 requirement for proper bone development, especially for calvarial ossification (Humbert et al., 2000; Miller et al., 2010). These generated craniofacial defects are thought to contribute to the aberrant accumulation of proteinaceous secretions in nasal cavities leading to lethality (Humbert et al., 2000). Thirdly, recall proliferation of CD8+ T-lymphocytes, which participate in viral infections control, is impaired (Bancos et al., 2009).

Aside from its role during brain development, implication of E2F4 in neuronal differentiation has been strengthened by in vitro studies using the pheochromocytoma line (PC-12 cells), which show reduced neuronal differentiation following E2F4 depletion and accelerated NGF-induced neuronal maturation with E2F4 overexpression (Persengiev et al., 1999). Other laboratories have also documented a role for E2F4 in repressing adipocyte differentiation independently of its cell cycle regulation properties but through PPARγ repression, a primordial factor in adipogenesis (Fajas et al., 2002; Landsberg et al., 2003; Tseng et al., 2005).

E2F4 also appears to play a critical role in rapid renewing tissues. In the gut, E2F4 is highly and preferentially expressed in the nucleus of proliferative cells (Dagnino et al., 1997b; Deschenes et al., 2004; Garneau et al., 2009). Loss of E2F4 in the small intestine results in a significant decline in proliferative zones (crypts) and a shortening and a reduction in the number of intestinal villi (Rempel et al., 2000). The role of E2F4 in maintaining intestinal homeostasis is also reinforced by the fact that it is overexpressed in the nucleus of colorectal cancer cells, contributing to hyperproliferation (Mady et al., 2002; Garneau et al., 2007; Garneau et al., 2009). In human and mouse epidermis, E2F4 is expressed in the basal and the immediately suprabasal cells, fading in upper cell layers (Dagnino et al., 1997b; Paramio et al., 2000; Wang et al., 2000; D'Souza et al., 2001). Although E2F4 has been reported to contribute to cell cycle arrest and differentiation in keratinocytes (Iavarone and Massague., 1999; Paramio et al., 2000), overexpressed E2F4 increases keratinocyte proliferation leading to hyperplasia and to an increased response to a two-step skin carcinogenesis assay (Wang et al., 2000; Wang et al., 2001). Finally, E2F4-deficient mice display a marked macrocytic anemia caused by impaired cell cycle progression and proliferation of fetal erythroid precursors also accompanied by maturation defects in multiple other hematopoietic lineages (Rempel et al., 2000; Kinross et al., 2006; Zhang et al., 2010).

In addition to the roles identified in proliferation, differentiation and development, other unconventional functions have been attributed to E2F4. Indeed, E2F4 binds various genes having functions in mitochondrial biogenesis, metabolism, cytoskeleton and mRNA processing (Cam et al., 2004). Moreover, E2F4 is thought to regulate the expression of certain miRNAs (Lee et al., 2011), control DNA repair (Ren et al., 2002; DuPree et al., 2004; Bindra and Glazer, 2007; Crosby et al., 2007; Dominguez-Brauer et al., 2009; Hegan et al., 2010; Lee et al., 2011), control survival in certain specific cell contexts (Chang et al., 2000; Wang et al., 2000; Ebelt et al., 2005; Garneau et al., 2007; Yang et al., 2008; Lee et al., 2011) as well as regulate aging and senescence (Iakova et al., 2003; Litovchick et al., 2011; Martin et al., 2011). Lastly, although the majority of E2F4 binding sites are located near transcription start sites and contribute to direct activation or repression of transcription (Lee et al., 2011; Lo et al., 2011), many sites are frequently localized more than 20 kb away from any annotated transcription start sites, suggesting that E2F4 can also act as a long-range transcriptional regulator (Lee et al., 2011).

### Homology

E2F4 is more related to E2F5 (69% identity, 80% similarity) than E2F1-3 (between 36% and 40% identity, between 52% and 60% similarity) (Sardet et al., 1995). In the E2F4 DNA-binding domain, residues mediating contact with DNA are conserved throughout the E2F transcription factor family (Zheng et al., 1999). Seventy-five percent of the DP interaction interface is identical within E2F transcription factors family (Zheng et al., 1999) and the pocket protein interaction domain of E2F4 has 48% homology with the Rb-interacting domain of E2F1 (Beijersbergen et al., 1994).

## Implicated in

### Colorectal cancer

**Note**

**Summary:** Mutation (AGC repeat) (Yoshitaka et al., 1996; Souza et al., 1997; Ikeda et al., 1998; Moriyama et al., 2002); Increased expression (Mady et al., 2002; Garneau et al., 2009).

Many studies have reported the presence of mutations in E2F4 AGC trinucleotide repeats in colorectal cancer bearing microsatellite instability (MSI). The more frequent mutations observed are the deletion or the addition of a trinucleotide AGC and the deletion of 7 trinucleotides (Yoshitaka et al., 1996; Souza et al., 1997; Ikeda et al., 1998; Moriyama et al., 2002). Furthermore, Takashima et al., 2001, studied the impact of E2F4 mutations and observed an increase in nuclear expression, in transcriptional activity as well as in proliferation rate of fibroblasts overexpressing these mutants.

### Gastric carcinoma

**Note**

**Summary:** Mutation (AGC repeat) (Kim et al., 1999; Ogata et al., 2001).

Kim et al., 1999, analyzed 56 gastric adenomas and 167 gastric carcinomas and found that frameshift mutations in E2F4 were more frequent in gastric adenomas than in carcinomas.

### Bladder cancer

**Note**

**Summary:** Amplification of chromosome arm 16q (Yu et al., 2001).

Comparative genomic hybridization (CGH) analysis revealed amplifications of chromosome arm 16q in 6/12 human transitional cell carcinoma (TCC) lines (more frequent in low-grade tumors) (Yu et al., 2001).

### Hepatocellular carcinoma (HCC)

**Note**

**Summary:** LOH on chromosome 16 (Sakai et al., 1992).

Using restriction fragment length polymorphism (RFLP) analysis on sixty-eight HCC specimens and their corresponding non-tumor liver tissues, a loss of heterozygosity was frequently observed on chromosome 16: specifically the MT2 locus at 16q21-22.1 (15%) and the HP locus at 16q22.1-22.2 (39%) (Sakai et al., 1992).

### Breast cancer

**Note**

**Summary:** Deletion of chromosome arm 16q22 or LOH (Dorion-Bonnet et al., 1995; Iida et al., 1997; Cleton-Jansen et al., 2001); Decreased expression (Ho et al., 2001); Increased nuclear expression (Rakha et al., 2004; Rakha et al., 2005).

Loss of chromosomal material at 16q22.1 is one of the most frequent genetic aberrations found in breast carcinogenesis suggesting the presence of a tumor suppressor gene (TSG) at this region (Dorion-Bonnet et al., 1995; Iida et al., 1997; Cleton-Jansen et al., 2001).

E2F4 is one of the candidate genes localized in this region and therefore was analyzed in different studies.

Ho et al., 2001, studied E2F4 protein expression in 10 primary breast carcinomas and 10 metastatic nodal tissues. The authors found a lower E2F4 protein expression in 7/10 primary breast carcinomas and in all (10/10) metastatic nodal tissues when compared to corresponding normal breast tissues. No tumor-specific mutation was detected, but polymorphisms were identified in the polyserine tract of E2F4 (3/11). Ho et al., 2001, further suggested that E2F4 is likely to function as a tumor suppressor in breast cancer.

Another group published two complementary and more in-depth studies arguing against the role of E2F4 as a TSG in breast cancer. Indeed, Rakha et al., 2005, used a Multiplex Amplifiable Probe Hybridization (MAPH) method to measure DNA copy-number at chromosome arm 16q22.1 in forty-nine invasive lobular, low-grade invasive ductal or tubular breast carcinoma samples. No correlation was detected between the expression of E2F4 with its gene's copy number.

Likewise, no significant loss or decrease in E2F4 protein levels was observed in malignant tissues. However, the authors did describe a correlation between increased nuclear expression of E2F4 and tumors with higher histological grade and positive lymph node disease whereas E2F4 was expressed in both the nuclei and cytoplasm in normal mammary epithelial cells, thus suggesting an oncogenic rather than a tumor suppressor role for this factor in breast cancer.

The same group (Rakha et al., 2004) also analyzed 265 breast carcinomas for E2F4 protein expression and found a correlation between increased nuclear expression of E2F4 and indicators of poor prognosis including larger tumor size, grade 3 lesions, lymph node stage and poorer Nottingham prognostic index group.

Increased E2F4 expression was also seen in association with the development of recurrent disease, distant metastasis and poorer outcome including poorer overall survival time and shorter disease-free interval.

### Prostate cancer

**Note**

**Summary:** Increased expression (Waghray et al., 2001).

Using serial analysis of gene expression (SAGE), Waghray et al., 2001, found that E2F4 was overexpressed by more than five-fold in prostate tumor tissues compared to the normal surrounding tissues.

Immunohistochemistry analysis further revealed strong E2F4 staining in epithelial cells of tumor glands as opposed to weak to no staining in normal glands.

### Hematological malignancies

**Note**

**Summary:** Mutation (AGC repeat) (Komatsu et al., 2000).

Komatsu et al., 2000, analyzed nine childhood acute lymphoblastic leukemia (ALL) samples, five acute myelocytic leukemia (AML) samples and ten adult T-cell leukemia (ATL) samples: frameshift mutations were found in E2F4 trinucleotide AGC repeats in 20% of ATL samples (3 AGC codon insertions) and in 11% of childhood ALL samples (6 AGC codon deletions).

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