C/EBPs

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CCAAT-enhancer-binding proteins (C/EBPs) is a family of six structurally homologous transcription factors that promote the expression of genes involved in different cellular responses, such as proliferation, growth, and differentiation. These transcription factors control the differentiation of several cell types, and have key roles in regulating cellular proliferation, through interaction with cell cycle proteins. The molecular structure of C/EBPs and their ability to interact with a multitude of factors determine their complex functions in different cells. In fact, C/EBPs can be activated or inhibited by a variety of intracellular or extracellular signals. In addition, post-translational modifications and interaction with other proteins can regulate their expression and activity in a complex manner. C/EBPs can activate or repress several classes of genes implicated in cell differentiation, metabolism, inflammation, and immune response. Moreover, C/EBPs play an important role in cancer progression and metastasis, showing both pro-oncogenic and onco-suppressor functions. Interestingly, the same isotype of C/EBP can exhibit both of these opposite functions. This “Janus” role of C/EBPs in cancer could depend on their particular position at the crossroads between proliferation and differentiation. Specific conditions such as cell type, microenvironment, type of heterodimerization, or interaction with different regulatory proteins can tip the balance towards pro- or anti-oncogenic action.

Keywords: C/EBP ; cancer ; tumor promoter ; tumor suppressor

1. Introduction

11. C/EBPs Structure and Isoforms

C/EBPs are constituted by different functional and structural components, which include a C-terminal leucine-zipper (a basic DNA-binding region) and in the N-termini of most C/EBP proteins (regions that act as transactivating and regulatory domains).

The leucine zipper is a highly conserved protein segment with a periodic repetition of leucine residues at every seventh position over a distance covering eight helical turns. The polypeptide segments containing these periodic arrays of leucine residues form a continuous α-helix that can dimerize through formation of a coiled-coil structure involving paired contacts between hydrophobic leucine zipper domains. Dimerization through the leucine-zipper leads to formation of homo- and heterodimers, which then bind with their two basic regions to DNA-sequences in the promoter/enhancer regions of a variety of genes. The basic residues interact in the major groove of the DNA, forming sequence-specific interactions [3].

The dimerization and the localization of leucines are critical for the DNA binding to the basic region. The basic regions of C/EBPs show a high binding affinity for (G/A)TTGCG(T/C)AA(T/C) or, broadly, the promoter CCAAT box sequence [4]. The N-termini of the C/EBP proteins are quite divergent, except for three short sub-regions that are conserved in most members and that represent the activation domains. The N-termini of some C/EBP proteins also contain regulatory domains that are conserved in most members [5]. Once bound to DNA, C/EBPs can recruit co-activators in their activation domains that, in turn, can open up a chromatin structure or recruit basal transcription factors that stimulate transcription.

The C/EBP family consists of six structurally and functionally homologous transcription factors—C/EBPα, C/EBPβ, C/EBPδ, C/EBPγ, C/EBPε, and CHOP. Apart from C/EBPγ and CHOP that are expressed ubiquitously, the other isoforms of C/EBP are distributed in specific tissues. For example, C/EBPα is prevalently expressed in the adipose tissue, blood mononuclear cells, liver, intestine, lung, adrenal gland, blood, nervous system, and placenta; C/EBPβ is expressed in the liver, adipose tissue, myelomonocytic cells, intestine, lung, spleen, nervous system, and kidney; C/EBPδ in the adipose tissue, myeloid cells, lung, nervous system, and intestine; and C/EBPε is expressed in myeloid and lymphoid cells.

1.2. C/EBPs Functions

C/EBPα plays a main role during the late phases of differentiation of pre-adipocytes. Both C/EBPβ and C/EBPδ mRNA are induced during the mitotic expansion of pre-adipocite cells. When pre-adipocytes exit, the cell cycle begins to express C/EBPα, which is then followed by the induction of adipocyte-specific markers [6]. It seems that C/EBPα expression is activated by the binding of C/EBPβ and C/EBPδ, with the C/EBPα promoter. C/EBPs are also implicated in myeloid differentiation [1]. Binding sites for the C/EBPs (α, β, δ, and ε) are present in the promoter regions of numerous genes that are expressed in myeloid cells. The expression of C/EBPα is relatively high in early myeloid progenitors and decreases during granulocytic differentiation. On the other hand, C/EBPε, is preferentially expressed during granulocytic differentiation, whereas C/EBPβ is up-regulated during macrophage differentiation. C/EBP proteins also have a role in the differentiation of hepatocytes, mammary, epithelial cells, ovarian luteal cells, keratinocytes, neuronal cells, and intestinal epithelial cells [1]. C/EBPs are implicated in the control of metabolism and in inflammation, as shown by the identification of binding sites for the C/EBPs, in the regulatory regions of a battery of genes involved in the inflammatory response, including those coding for cytokines and their receptors, acute-phase plasma proteins, and components of signal transduction pathways. Moreover, C/EBPα, C/EBPβ, and C/EBPδ were shown to be widely expressed in the mammalian nervous system and these seemed to be involved in the memory process [7]. Finally, most members of the C/EBPs family are implicated in the control of cell cycle and are involved during cancer progression, showing both tumor promoter and tumor suppressor activities.

2. C/EBPs and Cancer

C/EBPs are considered tumor suppressor factors for their ability to block cell growth, and for their role in cellular response to DNA damage. However, C/EBPs can elicit completely opposite effects on cell proliferation and cancer development, depending on the cell-type and the isoform present. They have also been described as both tumor promoters and tumor suppressors. This “Janus” role has been observed for all members of the C/EBP family (Table 1).

Table 1. Molecular mechanisms of tumor suppressor and the tumor promoting activity of each C/EBP family member.

C/EBP Type	Tumor Suppressor Activity	Tumor Promoting Activity	References
C/EBPα	Phosphorylated form at Ser 190 (193).	Dephosphorylated form at Ser 190 (193). Mutation of Ser 193 to Ala.	[8][9]
C/EBPβ	Phosphorylated isoform. β:β homodimers. Compartmentalization in perinuclear cytoplasm. Low LIP/LAP ratios.	Dephosphorylated isoform. β:γ heterodimers. Compartmentalization in peripheral cytoplasm. High LIP/LAP ratios.	[10][11][12][13][14][15][16][17]
C/EBPδ	Downregulation of cyclin D/E, C-Myc and upregulation of P27CIP2 in the early stages of tumor development.	Increasing translational activity of HIF-1α in breast cancer metastasis. Overexpression of HIF-1 α and downregulation of FBXW7α in glioblastoma. Overexpression of IGF-1 and PDGFA-R in cultured osteoblasts.	[18][19][20][21][22][23][24]
C/EBPγ	Inability to suppress C/EBP-mediated growth arrest in hepatoma cells. Inability to suppress C/EBPα growth arrest in different cell lines.	Inhibition of cellular senescence through heterodimerization with C/EBPβ.	[25][26]
C/EBPε	C/EBP-ε^{32} and C/EBP-ε^{30} isoforms are transcriptional activators that cause exclusively eosinophil differentiation. No specific effects on cancer.	C/EBP-ϵ^{27} is an inhibitor of GATA-1 inhibits eosinophil differentiation promoting granulocyte-macrophage differentiation. C/EBP-ε acts as a dominant-negative regulator. No specific effects on cancer.	[27][28][29]

C/EBP Type	Tumor Suppressor Activity	Tumor Promoting Activity	References
CHOP	Induction of apoptosis by inhibition of Bcl-2 and upregulation of Bim, PUMA, DR5 and p21.	Activation of MDSCs. TH17 propagation that promotes tumor growth via IL6-STAT3 pathway. Fusion with FUS/TLS or EWS by genomic rearrangement.	[30][31][32][33][34][35][36][37][38][39]

2.1. C/EBPα

C/EBPα was the first member of C/EBP family cloned. It is expressed prevalently in post-mitotic cells and it seems implicated in regulation of cell-cycle exit and differentiation in adipocytes, hepatocyte, myeloid cells, and other tissues [40][41][42][43][44]. C/EBPα induces cell growth arrest by blocking the association of CDKs with cyclins [45] and stabilizing the CDK2-p21 inhibitory complex [46]; moreover, C/EBPα can directly inhibit the activity of free CDK2/CDK4 [45]. In addition, C/EBPα might also associate with E2F complexes and convert them into repressors capable of inhibiting the S-phase gene transcription [47]. In this context, C/EBPα plays a role in the cellular response to DNA damage induced by extrinsic DNA-damaging agents. In fact, the C/EBPα gene is a p53-regulated DNA damage-inducible gene in keratinocytes and it is an important link between UVB-induced DNA damage and cell cycle arrest in epidermal keratinocytes [48].

Two isoforms of the C/EBPα are generated from its mRNA by a ribosomal scanning mechanism—the full-length 42 kDa C/EBPα (p42), which is implicated in the transcriptional activation of adipocyte genes and the 30-kDa isoform (p30). p30 is an alternative translation product initiated at the third in-frame methionine codon of the C/EBPα mRNA. Unlike p42, which inhibits cell proliferation, p30 seems not to exert an antiproliferative function [49].

For its ability to induce growth arrest contributing to the terminal differentiation of several cell types, and for its role in the cellular response to DNA damage, C/EBPα is considered a potent tumor suppressor factor. Deregulation of its expression can predispose to different malignancies, especially hematological neoplasms.

In fact, C/EBPα plays a main role in hematopoiesis. It is essential for myeloid differentiation and it has been implicated in regulating self-renewal of fetal liver and adult hematopoietic stem cells. Disruption of C/EBPα blocks the transition from myeloid committed stem cells to granulocyte/monocyte progenitors, leading to the loss of mature granulocytes [50]. In adult hematopoietic stem cells, the loss of C/EBPα causes increased proliferation, an increased number of functional long-term hematopoietic stem cells, and advanced repopulating ability [51]. N-Myc seems to be the downstream target of C/EBPα in hematopoietic stem cells. In fact, transcriptional repression of N-Myc by C/EBPα maintains the hematopoietic stem cells in a quiescence status [52]. Downregulation of C/EBPα plays a role in leukemogenesis. C/EBPα function is indeed frequently abrogated in acute myelogenous leukemias (AML) and oncogenes, such as AML1-ETO, BCR-ABL, or FLT3-ITD. This can downregulate or suppress C/EBPα, causing a block in myeloid differentiation and thereby inducing leukemogenesis [53][54][55][56]. Deregulation of the C/EBPα expression was also reported in a variety of additional human tumors, including breast and lung cancer [3

Discordant results were observed in hepatocellular carcinoma (HCC) and in hepatoblastoma (HBL). Tomizawa et al. examined the expression level of the C/EBPα and C/EBPβ genes between tumor and non-tumorous tissues of the same hepatocellular carcinoma patients, with quantitative real-time polymerase chain reactions showing that the expression of both the C/EBPα and C C/EBPβ genes was downregulated in the majority of the tumor specimens compared to the corresponding non-tumorous tissues. Patients whose expression of either C/EBPα or C/EBPβ was higher in tumors than non-tumorous tissues, survived longer than those whose expression was lower in tumors [57]. Similar results were obtained by Tseng et al. in a retrospective cohort study on 50 HCC patients. They observed that a reduced expression of the C/EBPα protein in HCC was associated with an advanced tumor stage and shortened patient survival [58]. In contrast, Lu et al. demonstrated that C/EBPα overexpression was correlated with poorer HCC overall survival [8]. Moreover, upregulation of C/EBPα was described in hepatoblastoma (HBL) [9]. These data suggest that C/EBPα might act as both a tumor suppressor and tumor promoter factor in liver cancers. These opposite functions might be correlated to a different posttranslational phosphorylation switch of C/EBPα. Phosphorylation of C/EBPα at ser190 (ser193 in mice homologue) is essential to maintain quiescence of hepatocytes through two pathways—inhibition of cdks and repression of E2F [59]. However, in liver tumor cells, the activation of the PI3K/Akt pathway blocks the growth inhibitory activity of C/EBPα, through the PI3K/AKT-protein phosphatase 2 (PP2A)-mediated dephosphorylation of C/EBPα on Ser 193 [59]. Dephosphorylated C/EBPα is unable to interact with and inhibit cdks and E2F with the consequent promotion of cell growth. Mutation of Ser 193 to Ala also abolishes the ability of C/EBPα to cause growth arrest, as it prevents the interaction of C/EBPα with cdk2 and E2F-Rb complexes [59]. Cast et al. investigated liver cancer in the mouse model

C/EBPα-S193A, in a large cohort of human HBL samples, and in Pten/p53 double knockout mice, and found that these cancers were characterized by an elevation of C/EBPα, which was dephosphorylated at Ser190/193. They found that dephosphorylated C/EBPα creates preneoplastic foci with cancer stem cells that give rise to HCC and aggressive HBL [60]. Therefore, conversion of the tumor suppressor C/EBPα into an oncogenic isoform can create preneoplastic foci where hepatocytes dedifferentiate into cancer cells, giving rise to liver cancer expressing high levels of mutated C/EBPα.

The development of liver cancer can also be determined by degradation of C/EBPα. Carcinogens such as diethylnitrosamine/phenobarbital (DEN/PB) can induce specific degradation of the phosphorylated isoform of C/EBPα, through activation of the ubiquitinproteasome system (UPS). The mechanism of the UPS-mediated elimination of C/EBPα during carcinogenesis involves elevated levels of gankyrin (an oncogenic protein that was found to interact with the phosphorylated form of C/EBPα) and targets it for UPS-mediated degradation [61][62][63].

2.2. C/EBPβ

C/EBPβ, which initially denominated the nuclear factor for IL-6 (NF-IL6), was first described in 1990 as a factor binding to the interleukin 1 (IL-1)-responsive element in the IL-6 promoter and showed high C-terminal homology to C/EBPα [64]. Subsequent knockout experiments revealed that the C/EBPβ knockout mice were viable but exhibited female sterility, defective mammary epithelial differentiation, and impaired immune function [1].

In fact, C/EBPβ is implicated in cell differentiation and in the regulation of genes involved in immune and inflammatory responses, such as *IL-6*, *IL-4*, *IL-5*, and *TNF-alpha* genes [65][66][67][68]. Moreover, it is critical for macrophage and B-cell differentiation [69][70]. C/EBPβ can activate genes that have specific roles in the nervous system—it can interact with an element of the preprotachykinin-A promoter, facilitating the substance P precursor gene transcription and with the promoter P2 of the choline acetyltransferase gene, inducing the biosynthesis of acetylcholine [71]. C/EBPβ also seems to be implicated in activation of genes coding for proteins that confer multidrug resistance to the cells regulating the liver expression of the MRP2 gene and activating the MDR1 gene in the MCF-7 cells [72][18].

The implication of C/EBPβ in cancer and tumorigenesis is more complex than that of C/EBPα. Many biological properties of C/EBPβ are similar to those of C/EBPα (since it blocks proliferation, promotes differentiation, and suppresses tumorigenesis), and similarly to C/EBPα. C/EBPβ is able to suppress cell proliferation through repression of the E2F target genes, thereby causing cellular senescence [73].

Expression of oncoproteins in primary cells often provokes cellular senescence, which is a permanent state of cell growth arrest that acts as a tumor suppressor mechanism. This cytostatic response, termed oncogene-induced senescence (OIS) is implemented through induction of the p19Arf-p53 tumor suppressor pathway and CDK inhibitors, such as p16Ink4a and p21CIP1, which activate Rb-dependent checkpoints [74][75][10]. C/EBPβ exerts anti-oncogenic effects because it is required for OIS.

Nevertheless, C/EBPβ can also exert pro-oncogenic effects [11]. These opposite activities depend on different causes, such as homo- or -heterodimerization, presence of inhibitors or presence of different isoforms of C/EBPβ.

C/EBPβ is maintained in a latent state by several auto-inhibitory elements that suppress its DNA-binding and transactivation functions [5][12]. Oncogenic stimuli, such as oncogenic RAS, can activate the RAF–MEK–ERK pathway that causes the phosphorylation and activation of the C/EBPβ [12][13]. Oncogenic Ras also increases the ratio of C/EBPβ homodimers with respect to C/EBPβ:C/EBPγ heterodimers, through a mechanism involving phosphorylation on leucine zipper residue Ser273 by p90Rsk kinases [14]. The homodimeric form of C/EBPβ contributes to the Ras-induced cell-cycle arrest and senescence in primary cells, whereas β:γ heterodimers actively promote cell growth [14]. Moreover, in immortalized and transformed cells, Ras-induced post-translational activation of C/EBPβ is inhibited by the 3′ untranslated region (3′UTR) of its mRNA, suppressing the cytostatic and pro-senescence functions of C/EBPβ [15]. The 3′UTR inhibitory effect was mapped to a region bearing the G/U rich elements (GREs). Moreover, an AU-rich element (ARE) and the ARE/GRE-binding protein HuR are required for 3′UTR inhibition. These components act by directing C/EBPβ transcripts to the peripheral cytoplasm, excluding them from a perinuclear region where the C/EBPβ kinases ERK1/2 and CK2 reside in RAS-transformed cells. In this location, newly translated C/EBPβ is uncoupled from RAS signaling and fails to undergo phosphorylation and activation by the RAF–MEK–ERK pathway. Thus, the intracellular site of the C/EBPβ translation is critical for RAS-induced activation via effector kinases such as ERK. Interestingly, 3′UTR inhibition and C/EBPβ mRNA compartmentalization are not observed in primary mouse and human fibroblasts [16].

The pro- and anti-tumorigenic activities of C/EBPβ can be, in part, determined by the presence of different isoforms of C/EBPβ. In fact, C/EBPβ is expressed as three isoforms with distinct activities—full-length LAP1 (or C/EBPβ p38 or Liver Activating Protein* (LAP*)), LAP2 (or C/EBP-β p32) that are lacking 21 amino acids (23 in human proteins) from the N-terminus, and Liver Inhibitory Protein (LIP or C/EBPβ p20) that lacks the whole activation domain (TAD) and acts as a transcriptional repressor. LAP1 is generally involved in terminal differentiation of cells, whereas LAP2 and LIP promote cell proliferation and tumor progression [17]. The proper ratios of the three isoforms are critical for normal cell growth and development. For example, an excess of the LIP isoform was observed in tumor cells that evade the growth inhibitory action of TGFβ. In normal cells, TGF-β block the cell cycle at the G1 phase, induce differentiation, or promote apoptosis. C/EBPβ is essential for TGFβ induction of the cell cycle inhibitor p15 INK4b and repression of C-Myc in human epithelial cells. These cytostatic responses can be missing in metastatic breast cancer cells. This loss seems to depend on the excess of the C/EBPβ inhibitory isoform LIP [19]. An excess of LIP was also observed in FLT3-ITD signal transduction. FLT3 is a cytokine receptor involved in cell growth and apoptosis regulation. Mutations that cause constitutive activation of the FLT3 receptor are frequent in acute myelogenous leukemia (AML) patients. The most frequent FLT3-mutations in AML are internal tandem duplications (ITDs) that lead to a constitutive activation of this receptor. In FLT3-ITD positive cells or when ITD sequences were inserted into the FLT3-wild type receptor, the LIP and LIP/LAP ratios were significantly increased, showing enhanced proliferation rates of AML cells. In addition, incubation of the FLT3-wild type cells with the FLT3 receptor ligand also elevated the LIP, LIP/LAP ratios, and proliferation [76].

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