The role of ATF-2 in oncogenesis

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Summary

Activating Transcription Factor-2 is a sequence-specific DNA-binding protein that belongs to the bZIP family of proteins and plays diverse roles in the mammalian cells. In response to stress stimuli, it activates a variety of gene targets including *cyclin A, cyclin D* and *c-jun*, which are involved in oncogenesis in various tissue types. ATF-2 expression has been correlated with maintenance of a cancer cell phenotype. However, other studies demonstrate an antiproliferative or apoptotic role for ATF-2. In this review, we summarize the signaling pathways that activate ATF-2, as well as its downstream targets. We examine the role of ATF-2 in carcinogenesis with respect to other bZIP proteins, using data from studies in human cancer cell lines, human tumours and mouse models, and we propose a potential model for its function in carcinogenesis, as well as a theoretical basis for its utility in anticancer drug design. *BioEssays* 30:314–327, 2008. © 2008 Wiley Periodicals, Inc.

Introduction

Tumourigenesis is considered to be a multistep process. These steps reflect genetic and epigenetic alterations that result in progressive conversion of a normal human cell into a highly malignant one. Animal models and human cancer studies indicate that tumour development proceeds through a process analogous to Darwinian evolution, in which ''successful'' genetic changes offer a growth advantage to normal cells, leading to their progressive transformation into cancer cells. For a cancer phenotype to develop, the normal cell progressively acquires certain capabilities such as self sufficiency in growth signals, insensitivity to anti-growth signals, unlimited replicative potential, evading apoptosis, sustained angiogenesis, as well as tissue invasion and metastasis.[1]

Since in situ studies in human material are not sufficient to elucidate the mechanisms underlying the sequential events of the carcinogenetic process, both animal and cancer cell line models have been recruited in order to complement the studies of human pre-malignant and malignant regions. Perhaps the most popular tumourigenesis study models are the mouse models. Mouse models have often been invaluable in understanding several topics of human carcinogenesis and, most importantly, in contrast to cell models, they provide in vivo rather than in vitro information for tumour development, metastasis and testing of anticancer drugs.[2]

Recent studies implicate the ATF/CREB family of transcription factors in cancer progression.[3,4] The ATF/CREB family is a large group of bZIP transcription factors which, despite their diverse physiological roles, all share the ability to respond to environmental signals and maintain cellular homeostasis.[5] Mounting evidence indicates a dynamic role for the ATF/CREB family member ATF-2 in various steps of the carcinogenetic process. The *ATF-2* (or *CREB-BP1*) gene is located on human chromosome 2q32 and encodes a 505 aa protein (see Fig. 1).[6] Studies on ATF-2-deficient mice reveal an essential role in skeletal and central nervous system development, and for maximal induction of genes with CRE sites.[7] Our previous work with the mouse skin carcinogenesis model has revealed a role of the ATF/CREB family member ATF-2 in growth and progression of mouse skin tumours.[8,9] In this review, we summarize the signals that activate ATF-2 as well as the downstream targets of ATF-2 and we examine its role in human tumours, cancer cell lines and animal models. We exploit these pieces of information to come up with a model for the action of ATF-2 in carcinogenesis. We also highlight possible strategies for targeting against ATF-2, for future *ATF-2*-based anticancer drug design.

ATF/CREB family proteins are implicated in cancer

The mammalian ATF/CREB family consists of sixteen cellular stress-responsive transcription factors, divided into six subgroups, according to their sequence similarity.[5,10–12] The common feature that all these proteins share is the bZIP element, through which they can both dimerize and bind to specific DNA sequences (see Fig. 2).[10–19] The bZIP element consists of a leucine zipper subdomain and a basic region subdomain that are connected by a short fork.[20] ATF/CREB family proteins not only homodimerize, but also selectively heterodimerize with each other.[21,22] However, they do

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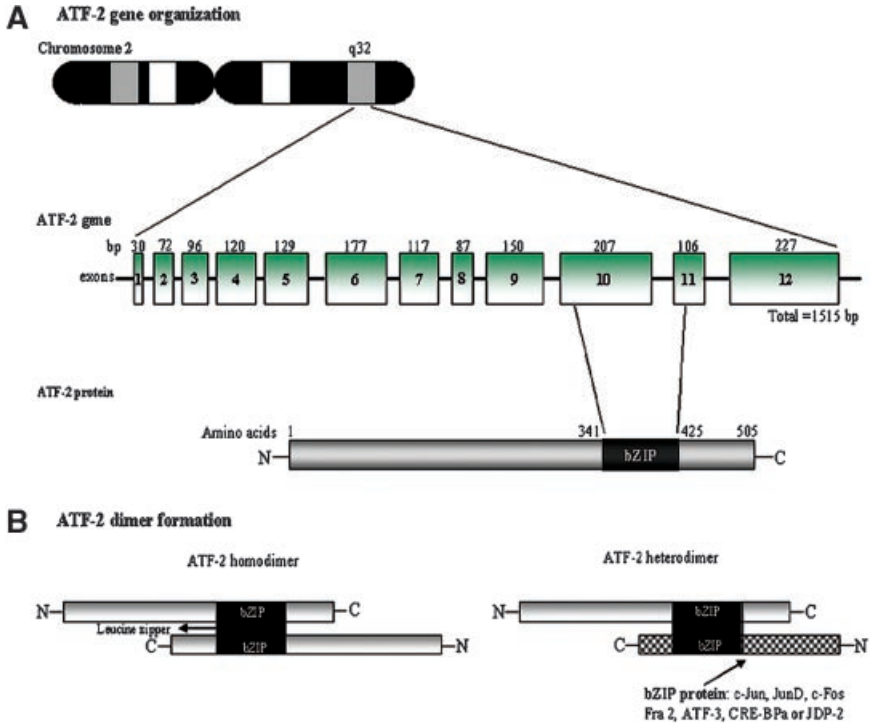

Figure 1. A: ATF-2 gene organization. The ATF-2 consists of 12 exons and it is translated to a 505 aa protein. The bZIP protein domain corresponds to parts of exon 10 and exon 11 of ATF-2 gene. **B:** ATF-2 dimer formation. ATF-2 forms homodimers or heterodimers with other b-ZIP proteins.

not share much similarity other than the bZIP domain and their ability to bind to the ATF/CRE consensus TGACGT$^{C}_{A}$$^{G}_{A}$.[21,23]

Many family members are positively associated with cancer progression. For example, ATF-1 acts as a survival factor for human melanoma cells[24] and promotes tumour invasiveness of thyroid papillary carcinoma.[25] ATF-4 overexpression offers drug resistance to human cancer cell lines.[26] CREB phosphorylation enhances tumour survival of human lung cancer cell lines.[27] ATF-6 overexpression is implicated in hepatocarcinogenesis,[28] while ATF-5 has been suggested to have an anti-apoptotic role.[29]

However, other members of the family present both positive and negative involvement in cell survival. On the one hand, ATF-3 overexpression promotes invasiveness of prostate tumour cells[30] but, on the other hand, it has been characterized as a putative anti-tumourigenic gene in ovary cancer.[31] There are also conflicting reports about the role of ATF-2 in cancer. Recent reports demonstrate that ATF-2 overexpression enhances cell proliferation both in human[4,32] and in mouse[9] cancer cell lines. In contrast are the findings of a report showing that reduced levels of ATF-2 predispose mice to mammary tumours.[33] This review, for the first time, sheds light on the actual role of ATF-2 in cancer.

Signals activating ATF-2

ATF-2 contains Sp1 elements and a CRE-like element in promoter region −50 to +90 that seems to be important for basal promoter activity;[34] however its activation is basically achieved through posttranslational modifications, upon stress stimulus. In unstimulated cells, ATF-2 is maintained in a transcriptionally inactive form by intramolecular interactions between its own activation domain and its bZIP domain.[35] In response to stress stimuli[36–42] the Ras-activated signal cascades p38 and JNK phosphorylate ATF-2 protein at amino acids Thr69 and Thr71.[36,37] The Ras effector pathway Raf-MEK-ERK also acts synergistically with the Ral-RalGDS-Src-p38 pathway in a two-step ATF-2 phosphorylation, where the former phosphorylates Thr71, while the latter phosphorylates Thr69.[43]

Phosphorylated ATF-2 forms dimers that bind to specific DNA sequences on target gene promoters, activating their expression. ATF-2 can form homodimers or selective heterodimers with other members of the ATF/CREB family (see Table 1)[10,12,16,19,21,44–52] and Fos/Jun family (see Table 2).[3,16,19,45,49,51–59] Typically, ATF-2 dimers bind preferentially to the 8-base palindromic CRE (cAMP Responsive Element) consensus sequence T$^{G}_{T}$ACGTCA on target gene promoters, that responds to elevated cAMP.[21,60] However, ATF-2 has also been shown to bind to other elements, such as

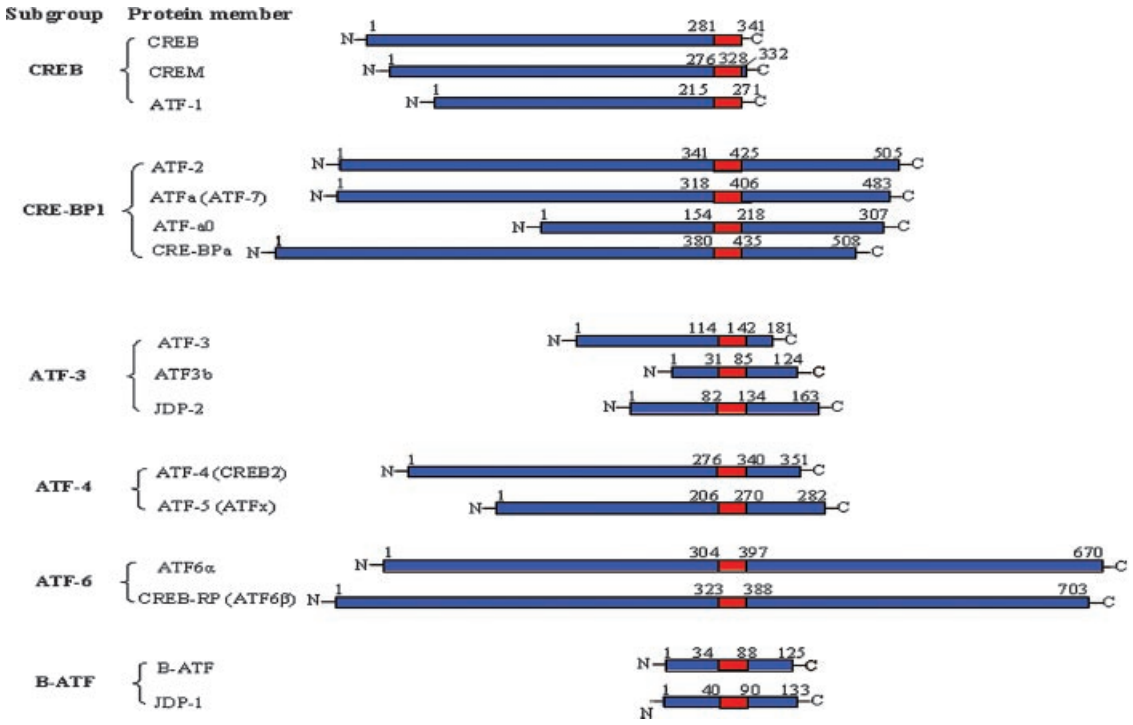

Figure 2. ATF/CREB family proteins. The protein members can be grouped in six subgroups, according to their sequence similarity. The red boxes indicate the bZIP domain. (See references 10–19 and GenBank Accession numbers: AAC60616, Q02930, AAC02258, P18848, AAD51372, AAB49921).

the AP-1 element,[61,62] the proximal element of *IFN-γ* promoter,[63] the stress-response element (StRE) of *ho-1* gene,[64] and the UV-Responsive Element (URE).[4]

Although only few Ras-promoted pathways lead to ATF-2 activation, the ability of ATF-2 to form homodimers and a wide range of selective heterodimers, as well as to bind to more than one type of responsive elements, may offer ATF-2 a certain degree of functional plasticity, resulting in the activation of a variety of target genes.

ATF-2 activates target genes involved in cancer

Activated ATF-2 complexes stimulate a broad spectrum of targets that are implicated in cancer (see Fig. 3):

Cell-cycle molecules

cyclin D1, an important gene for the integration of proliferative and antiproliferative signals during the G_1 phase of the cell cycle, possesses a CRE element within its promoter region. In mouse chondrocytes, *cyclin D1* is directly activated by ATF-2, while the levels of activation are reduced in ATF-2-deficient mice.[65] *cyclin D1* activation by ATF-2 has also been demonstrated in proliferating mouse melanoma cells.[66] Similarly, *cyclin A* plays an essential role in S phase progression. In mouse chondrocytes, serum-activated ATF-2 promotes Cyclin A expression by binding a CRE-responsive element located in the promoter of *cyclin A,* whereas the exact opposite is observed in ATF-2-deficient mice.[39]

Molecules related with invasion

The promoter of *MMP-2*, a matrix metalloproteinase capable of degrading all components of the extracellular matrix, possesses an ATF-2-responsive AP-1 element.[61] In addition, urokinase Plasminogen Activator (uPA), an enzyme necessary for the degradation of a variety of extracellular proteins that connect cells to the extracellular matrix, used as a prognostic marker in various malignancies,[67] responds to c-Jun–ATF-2 heterodimers that bind to an AP-1 element on its gene promoter.[62]

Growth factors/receptors—cytokines

Platelet-derived growth factor receptor α (PDGFR α) is a growth-regulatory protein that has been characterized as an ATF-2 target in mice. In cytotrophoblast cells from ATF-2 null mice placenta, PDGFR α is downregulated compared to ATF-2 wild-type mice.[68] In addition IL-8, a chemokine involved in epithelial–mesenchymal transition of colon carcinoma,[69] is responsive to p38-activated ATF2–c-Jun heterodimers that bind to AP-1 sites located in the *IL-8* promoter.[70]

Table 1. Dimer formation between ATF/CREB family members (intrafamily dimerization)

	ATF/CREB FAMILY MEMBERS															
	ATF-1	**ATF-2**	**ATF-3**	**ATF3b**	**ATF-4**	**ATF-5**	**ATF6α**	**ATF6β**	**ATFa**	**ATF-a0**	**B-ATF**	**CREB**	**CREM**	**CRE-BPa**	**JDP-1**	**JDP-2**
ATF-1	+[21]	−[21]	−[21]				−[46]					+[52]				
ATF-2	−[21]	+[21]	+[21]				−[46]					−[49]		+[16]		+[48]
ATF-3	−[21]	+[21]	+[21]	+[10]		−[51]						−[52]				
ATF3b			+[10]	+[10]												
ATF-4					−[47]	−[47]						+[49]				
ATF-5			−[51]		−[47]	−[47]										
ATF6α	−[46]	−[46]					+[50]									
ATF6β																
ATFa									+[12]	+[12]						
ATF-a0									+[12]	+[12]						
B-ATF											−[19]					
CREB	+[52]	−[49]	−[52]		+[49]							+[50]	+[44]			
CREM												+[44]				
CRE-BPa		+[16]												+[16]		
JDP-1															−[45]	
JDP-2		+[48]														+[45,48]

+: dimer formation.
−: dimer formation is not possible.

Table 2. Dimer formation between ATF/CREB and Fos/Jun family members (interfamily dimerization)

	JUN FAMILY PROTEINS			FOS FAMILY PROTEINS			
	c-Jun	**JunB**	**JunD**	**c-Fos**	**FosB**	**Fra-1**	**Fra-2**
ATF-1	−[53]			−[52]		−[53]	
ATF-2	+[53]		+[58]	+[53]		−[53]	+[55]
ATF-3	+[52]	+[52]	+[56,52]	−[51]/+[59]	+[59]	−[52]	
ATF3b							
ATF-4	+[53]		+[56]	+[53]	+[52]	+[53]	
ATF-5							
ATF6α							
ATF6β							
ATFa	+[57]	+[52]	+[52]	+[57]			
ATF-a0							
B-ATF	+[3,19]	+[3,19]	+[3,19]	−[19]			
CREB	−[49]			−[52]			
CREM	−[54]			−[54]			
CRE-BPa	+[16]						
JDP-1	+[45]						
JDP-2	+[45]						

+: dimer formation.
−: dimer formation is not possible.

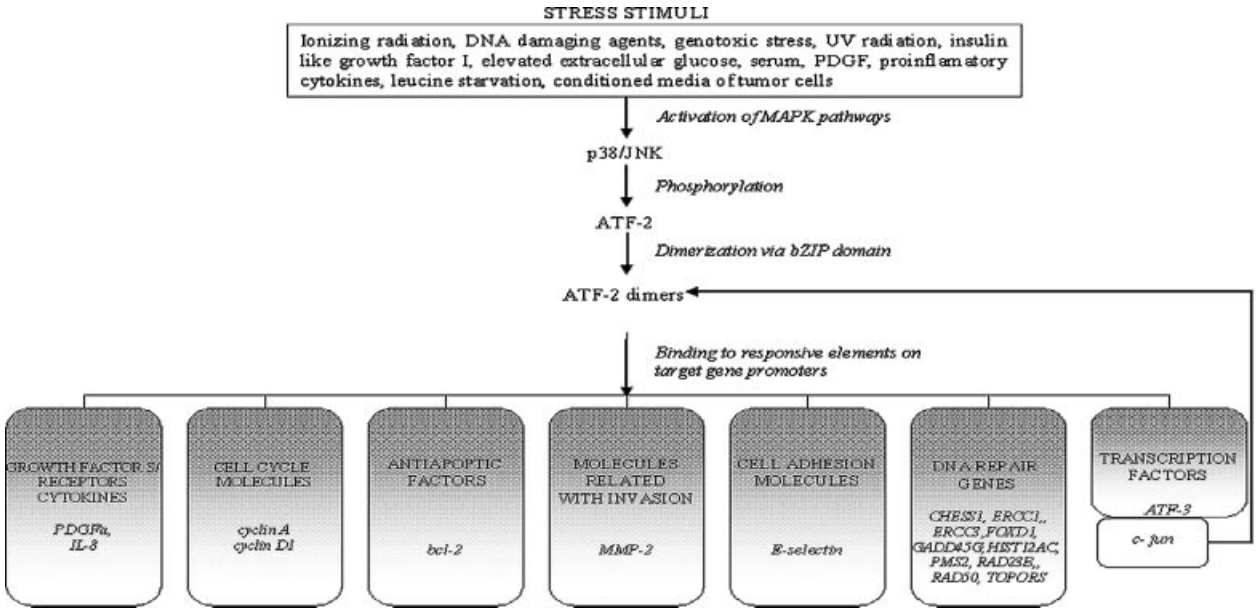

Figure 3. ATF-2 phosphorylation and activation of its downstream targets. Various stress stimuli activate the p38 or/and JNK pathways, which phosphorylate ATF-2. Phosphorylated ATF-2 forms homodimers or selective heterodimers, activating a variety of gene targets, many of which are implicated in processes that are deregulated in cancer, including *c-jun*. Newly synthesized c-Jun dimerizes with ATF-2, reinforcing transcription of ATF-2 gene targets.

Cell adhesion molecules

E-selectin seems to play a role in transendothelial migration of cancerous cells.[71] In endothelial cells, an ATF-2–c-Jun heterodimer is constitutively bound to a CRE-like sequence of the *E-selectin* promoter. Upon TNF-α stimulation, ATF-2 and c-Jun are phosphorylated by the MAP kinases JNK and p38 and co-operate with NF-κB in activating *E-selectin* transcription.[72]

Anti-apoptotic factors

bcl-2, one of the most-potent inhibitors of apoptosis, contains a CRE element responsive to ATF-2 in its promoter region.[73]

ATF-2 activates its bZIP-binding partners

c-jun transcription factor represents a special gene target of ATF-2, as it is both a target and a co-operator of ATF-2. c-Jun is a bZIP protein, which belongs to the Jun protein family. Through its bZIP domain, it forms either homodimers or heterodimers with other members of the Jun family (c-Jun–JunB, c-Jun–JunD heterodimers), as well as heterodimers with Fos family members. The dimers that Jun members form are called AP-1 proteins and bind to the AP-1 element located in numerous genes (reviewed in Ref. 123). Jun–Jun and Jun–Fos dimers activate transcription of target genes by binding to the 7-base classical pseudopalindromic AP-1 consensus sequence $T^{G}_{T}A^{C}_{G}TCA$, in contrast to the 8-base palindromic CRE consensus sequence $T^{G}_{T}ACGTCA$ that ATF-2–Jun heterodimers prefer to bind.[53,59,75]

The *c-jun* promoter carries two relatively similar AP-1-binding sites, jun1TRE (the one closest to the transcription start site) and jun2TRE (the one distal from the transcription start site), which resemble both ''classical'' AP-1 and ATF/CREB-binding sites. jun2TRE and, to a lesser extent, jun1TRE can bind c-Jun–ATF-2 heterodimers. In detail, a number of stress stimuli activate JNK–SAPK (Stress Activated Protein Kinases), which phosphorylate both ATF-2 (on Thr69 and Thr71) and c-Jun (on Ser63 and Ser73), thus enabling the heterodimer formation. The initial c-Jun–ATF-2 heterodimer enhances *c-jun* transcription via binding to the jun2TRE element.[76,77] c-Jun can also be de novo synthesized in response to other stimuli, such as PDGF, serum and phorbol esters, via the ERK pathway, however ATF-2 is not involved in this process; rather, ERK (Extracellular-Regulated Kinase) stimulates Jun–Fos dimer formation (reviewed in Ref. (78).

Moreover, ATF-2 activates the ATF/CREB family member ATF-3, a transcription factor with a highly controversial role in cancer. The *ATF-3* promoter contains both AP-1- and CRE-binding sites and responds to c-jun–ATF-2 complexes activated by the JNK–SAPK signal transduction pathway.[79] ATF-3 promotes cell cycle arrest and apoptosis by repressing *cyclin D1* transcription in Ras-stimulated mouse fibroblasts, a widely accepted model for studying Ras-stimulated tumourigenesis,[80] while it activates p53 by inhibiting its ubiquitination in the same study model.[81] In contrast to the above anti-proliferative role, overexpression of ATF-3 activates *cyclin D1* promoter, when AP-1 and CRE elements of *ATF-3* promoter remain intact, thus enhancing *cyclin D1* expression in the Hepa 1–6 mouse hepatoma cell line.[82] Since it has not been clarified whether *ATF-3* transcription is activated via the AP-1 or/and the CRE element, the opposing effects of ATF-3 could be attributed to differential accumulation of these crucial elements in response to different stress stimuli, as well as to its combination with different bZIP-binding partners.

As described above, ATF-2 regulates transcription of ATF/CREB and Jun/Fos family members. Other ATF/CREB family members present similar behavior, since CREB activates *c-fos* transcription via a CRE element on *c-fos* gene promoter, while CREM antagonizes the activating function of CREB, by forming inactive CREB–CREM heterodimers.[44] Furthermore, both CREB and CREM dimers block *c-jun* transcription by binding to the TRE responsive element.[54] Perhaps, regulation of Jun/Fos proteins by ATF/CREB proteins represents a general mechanism of regulating bZIP proteins' selective dimerizations and interactions. It is possible that *ATF-2* per se is regulated by other members of ATF/CREB family, since it possesses two CRE-like binding sites, one of which is located in a region significant for basal promoter activity.[34] However, it is of note that, among ATF/CREB family members, only ATF-2 seems to have the ability to activate two of its bZIP partners (see Table 1, Table 2). This exclusive characteristic implies that ATF-2 is a potential dynamic modifier of bZIP interactions and may drastically affect bZIP protein interactions in oncogenesis.

Interactions of ATF-2 with c-Jun in oncogenesis

ATF-2–c-Jun seems to be a common dimer in oncogenetic processes. Promoter array hybridization experiments in the human breast carcinoma cell line BT474 revealed that, upon genotoxic stress and subsequent JNK activation, c-jun–ATF-2 dimers could target 121 genes, including 10 that were implicated in the DNA repair process, activating their expression (see Fig. 3). c-Jun and ATF-2 interacted with the targets as complexes with discrete compositions, either as homodimers or as heterodimers, with the most common composition being that of c-Jun–ATF-2 heterodimers.[41]

ATF-2 seems to act as an important modifier of the dynamic balance between c-Jun and the other members of the Jun family, JunB and JunD. Their interactions affect cell cycle progression since, upon Ras stimulation, c-Jun induces G_1–S transition by activating crucial cell cycle regulators (*cdk4/6, cyclin D1*), whereas JunB and JunD have opposite effects (reviewed in Ref. [74]) By inducing c-Jun expression or/and by forming selective heterodimers with c-Jun and JunD (see Table 2), ATF-2 is indirectly involved in this antagonism. Alterations in ATF-2 activity, either by active ATF-2 mutants[83] or dominant

negative ones,[84] could result in c-Jun level alterations and, eventually, deregulation of cell cycle progression.

The binding partner of c-Jun in the AP-1 complex seems to affect its behavior in carcinogenesis. The decision of a cell to proliferate, differentiate or die by apoptosis depends on the composition of the AP-1 complex, as well as the cell lineage, the differentiation stage, the microenvironment and the type of stimulus (reviewed in Ref. 85). For example, in the human leukemia cell line HL60, AP-1 complex components change during 1,25-dihydroxyvitamin D3-induced differentiation. Specifically, AP-1 components in the early stage of differentiation include c-Jun, ATF-2, FosB, Fra-1 and Fra-2, whereas AP-1 components in cells with a more-established monocytic phenotype include c-Jun, ATF-2 and FosB.[86] In addition, in vitro studies in chicken embryo fibroblasts have revealed that when ATF-2 is the partner of c-Jun, then growth-factor independence is induced, but not anchorage-independence growth, whereas the opposite exists when c-Fos is the partner of c-Jun.[87] Perhaps, dimerization of ATF-2 with c-Jun results in the activation of CRE-responsive genes rather than AP-1-responsive genes and to subsequent alterations in the processes in which these genes are involved. ATF-2 could modify AP-1 carcinogenetic effects, not only by regulating *c-jun* transcription, but also by forming selective heterodimers and changing AP-1 composition, thus re-orientating c-Jun to CRE targets rather than AP-1 targets. If this hypothesis holds true, a single nucleotide between the 7-base AP-1 and the 8-base CRE elements makes the difference!?

Interactions of ATF-2 with other bZIP proteins in oncogenesis

In many cases, together with ATF-2, other bZIP family members drastically affect ATF-2 regulatory function in many ways within the cancerous environment. First of all, other ATF/CREB family members seem to inhibit ATF-2 function, not through antagonism for binding to CRE-responsive genes, but by taking advantage of the selective dimerization characteristic, in order to inhibit its binding to target gene promoters. For example, the rat *cyclin A* CRE element responds to ATF-2 and JunD. Notably, ATF-4 co-expression suppresses activation of the *cyclin A* promoter by ATF-2 and Jun family members, implying that an antagonistic relationship exists between ATF-2 and ATF-4 for Jun binding, which possibly results in inhibition of ATF-2–JunD heterodimerization.[58] Consistent with this idea, JDP-2 forms inactive heterodimers with ATF-2 as well as c-Jun, thus suppressing CRE and AP-1 target gene transcription correspondingly.[45,48] This type of interaction seems to be general for bZIP proteins, rather than ATF-2 exclusive, because the ATF/CREB family member B-ATF competes with c-Fos for c-Jun binding, resulting in transcriptionally inert Jun–B-ATF heterodimer formation and reduced cell growth rate in mouse study fibroblast cells.[3]

An additional type of ATF-2 interaction with other bZIP proteins in oncogenesis could be the antagonism between bZIP dimers for binding to the same promoter region, leading to either activation or inhibition of gene transcription via the same binding site. For example, in the hepatocarcinoma cell line HepG2, ATF-2 is a positive regulator of *ApoCIII*, upon TNF-α stimulation, whereas the Jun family members (c-Jun, JunB and JunD) repress *ApoCIII* through the same promoter region in a dose-dependent manner.[88] Activation of *IFN gamma* (*IFN-γ*), a gene known to prevent the development of primary and transplanted tumours, represents a more complex paradigm: the *IFN-γ* promoter possesses a composite regulatory element (proximal element) responsive to the transcription factors ATF-1, ATF-2, c-Jun and other Jun proteins, CREB and Oct-1. In unstimulated T cells, CREB, ATF-1, ATF-2 and Jun family proteins, other than c-Jun, are present, whereas in stimulated T cells a shift to phosphorylated c-Jun–ATF-2 dimers occurs.[63] An antagonism for binding between bZIP dimers seems to take place on the *IFN-γ* proximal element, because CREB homodimers or/and CREB–ATF-1 heterodimers antagonize Jun dimers for binding to this element. Alteration of the balance among these molecules, as well as their unphosphorylated forms, upon stress stimuli, results in activation or inhibition of *IFN-γ* transcription.

Alternatively, multiple bZIP proteins, including ATF-2 may not antagonize, but co-operate for the transcriptional activation of a target gene. Notably, in the human hepatocarcinoma cell line Hepa, the *ho-1* gene, which catalyzes the first and rate-limiting step in heme catabolism, possesses a stress-response element (StRE), responsive not only to ATF-2, but also to a number of other bZIP proteins (ATF-3, FosB, JunB and JunD).[64] Multiple dimer complexes among the above mentioned proteins could act either synergistically or equivalently for the activation of this gene.

Finally, ATF-2-containing complexes may have the ability to select between different types of binding elements, resulting in the activation of different subsets of target genes and, therefore, to expression of different cancer cell phenotypes. Specifically, in apoptotic rat C6 glioma cells the complexes, which comprise phosphorylated c-Jun, phosphorylated ATF-2, JunB and JunD, trans-activate FasL promoters, while in pro-apoptotic cells different bZIP protein composition results in selection of AP-1/TRE promoters.[89]

Altogether, it seems that a fine-tuned network of interacting bZIP proteins, including ATF-2, rather than a specific homodimer or heterodimer is involved in response to cellular stress stimuli. Interactions between ATF-2 and bZIP proteins involve a) regulation of other bZIP proteins' transcription, (b) antagonism with other bZIP monomers for selective dimer formation, (c) antagonism between bZIP dimers for binding to the same responsive element (d) co-operation or equivalency between bZIP dimers for binding to the same responsive

element,and (e) selection of the responsive element according to the overall cellular bZIP content. Deregulation of one or more of these types of interaction might cause alterations in processes such as proliferation, differentiation and apoptosis, thus leading to acquisition of cancerous characteristics. Additional parameters in the cell environment, e.g. the type of stress stimulus and the cell type may also play a role in the overall ATF-2 effect in oncogenesis. It is possible that other bZIP proteins could act through one or more of these types of interaction during oncogenesis. However, the fact that ATF-2 is implicated in all these types of interaction that affect target gene regulation makes it a potential dynamic modifier of the bZIP proteins' balance and thus a major modulator of the cell fate (see Fig. 4).

The role of ATF-2 in various types of cancer

In vitro studies in human cancer cell lines, as well as in situ studies reveal a well documented activation of JNK–p38–ATF-2 axis in several cancer types.

Prostate cancer

Recently, a substantial number of prostate cancer patients have been examined for phosphorylated ATF-2 expression, as well as other downstream components of p38 pathway. Analysis of samples from normal prostate tissue, benign prostatic hyperplasia and advanced prostate cancer revealed that phosphorylated ATF-2 is overexpressed in benign prostatic hyperplasia and, more intensely, in prostate tumours. These observations suggest that phosphorylated ATF-2 enhances survival and cell proliferation, promoting prostate cancer progression.[32] A recent study in over 200 prostate cancer samples showed that the expression of glucocorticoid receptors (GR) are decreased or absent. Reconstitution of GR expression in prostate cancer cell line LNCaP resulted in decreased expression of MAP-kinases (MAPK) activity, such as p38, JNK/SAPK, Mek1/2 and Erk1/2, and subsequent downregulation of numerous transcription factors, including ATF-2.[90] Therefore, GR acts as a tumour suppressor, blocking ATF-2 in prostate cancer. Collectively, these data demonstrate a possible proliferative role for ATF-2 in prostate cancer.

Breast cancer

ATF-2 has been correlated with proliferation, invasion and migration, as well as resistance to DNA-damaging agents in breast cancer cell lines. In MCF-7 cells, treatment with growth factors (estradiol, spermine) led to enhanced p38-mediated

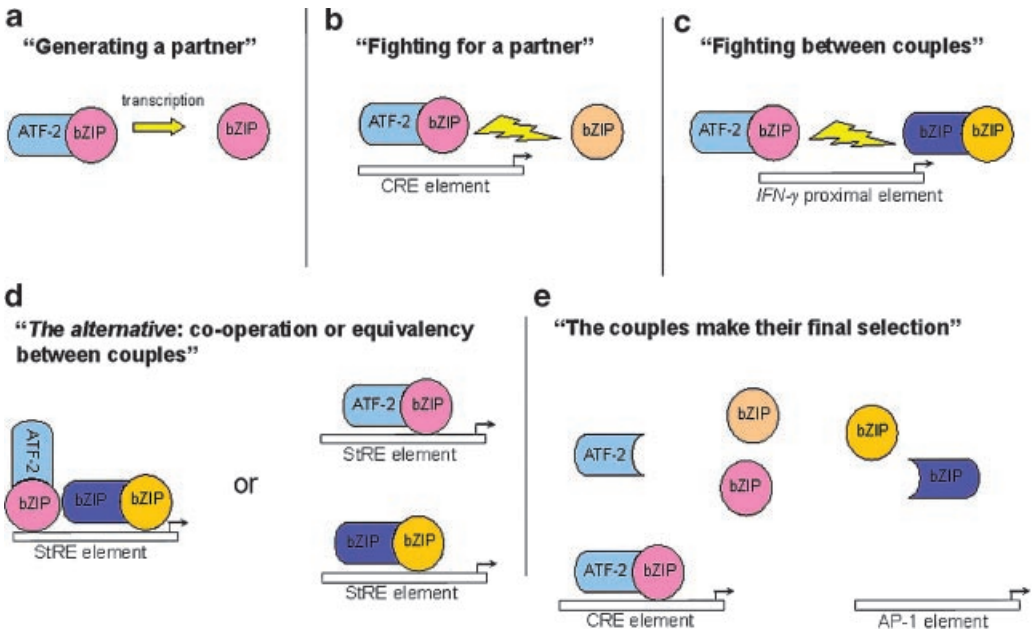

Figure 4. Possible types of interaction between ATF-2 and other bZIP proteins in oncogenesis: interactions between bZIP molecules in transcriptional and posttranslational levels lead to the ultimate selection of target genes that are crucial for cell proliferation, differentiation or apoptosis. ATF-2 is dynamically involved in all these interactions. **a:** Regulation of transcription of other bZIP proteins, **b:** antagonism for selective dimerization, leading to either transcriptionally inert or transcriptionally active dimers, **c:** antagonism between bZIP dimers for binding to the same binding element, leading to activation or inactivation of transcription of the corresponding gene, **d:** co-operation or equivalency between bZIP dimers for binding to the same responsive element, **e:** selection of the type of responsive element in crucial genes, according to the overall cellular bZIP content.

ATF-2 phosphorylation and subsequent increase in ATF-2 binding to the CRE element of *cyclin D1* promoter.[91] Moreover in MCF10A cells, p38-activated ATF-2 targets the AP-1 site of *MMP-2* and enables induction of invasive and migrative phenotypes.[61] Furthermore, in BT474 cells, resistance to DNA-damaging agents and, therefore, enhanced cell viability is mediated by increase in ATF-2 transcriptional activity. In contrast, inhibition of ATF-2 phosphorylation by a dominant negative mutant ATF-2 decreases cell viability, after treatment with DNA-damaging agents. However, in the latter cell line, ATF-2 phosphorylation is JNK-dependent, rather than p38-dependent.[40] As these experiments have been performed in vitro, this difference could be ascribed to the different molecular background of these cancer cell lines. A more-attractive hypothesis is that distinct signal transduction pathways (p38 or JNK) may activate ATF-2 in response to different stress stimuli, in different stages of tumour progression. The development of in vivo study models for breast cancer progression could clarify this issue.

In contrast, in MCF-7 cells, the promising anticancer agent DIM (3,3′-Diindolylmethane, derived from Brassica vegetables) activates both JNK and p38 pathways, resulting in c-Jun and ATF-2 phosphorylation, and subsequent augmentation of binding of the c-Jun–ATF-2 homodimers and heterodimers to the proximal regulatory element of *IFN-γ* promoter.[92] Perhaps the type of stress signal, as well as the presence of other ATF-2 bZIP partners are important in the selection of the kind of target gene, apoptotic or antiapoptotic, that is ultimately activated by ATF-2.

Leukemias

ATF-2 has been implicated in differentiation-related cell-cycle arrest and apoptosis in leukemia. In the human leukemia cell line HL60, a combination of antioxidants with 1,25-dihydroxyvitamin D3 induces JNK pathway activation in differentiating HL60 cells, followed by increased phosphorylation of c-Jun and ATF-2.[93] Furthermore in other leukemia cell lines, cepharanthine (CEP), a biscoclaurine alkaloid that induces caspase-dependent apoptosis, activates p38 and its downstream targets c-Jun and ATF-2, but not JNK.[94] As the overall functional impact of ATF-2 activation by these potential anticancer agents in leukemia cells is still obscure, identification of apoptotic or/and antiapoptotic genes activated by ATF-2 in these processes could provide more direct evidence for the ATF-2 role in leukemia.

Tumours of the nervous system

The balance between ATF-2 and c-Jun contributes to neuronal differentiation, cell survival and apoptosis during neuronal development. For example, in rat pheochromocytoma PC12 cells, a well-established model for studying neuronal differentiation, c-Jun protects the cells from ATF-2-mediated apoptosis, ensuring that the neuronal differentiation will be completed. Once the cells have been differentiated, ATF-2 acts as an executor of apoptosis.[95] Since the same processes are generally deregulated in cancer, one could assume that ATF-2 might also be implicated in neuroblastoma. Indeed, experimental data have correlated ATF-2 with nervous system tumourigenesis, indicating a possible proliferative role in this type of cancer. In SK-N-MC and SH-SY5Y human neuroblastoma cells, ATF-2 is potently activated by JNK pathway.[96] Furthermore, in neuroblastoma cells N2α-β, oncogenic Ras induces binding of ATF-2–CREB heterodimers to *cyclin D1* promoter, and subsequent cell proliferation. Thyroid hormone receptor represses Ras-induced transcriptional activity of CREB and ATF-2, in response to T3 thyroid hormone.[97] Further studies, focused on role of ATF-2 in neuroblastoma, could provide more information about the implication of this molecule in neuronal oncogenesis.

Hepatic cancer and lung cancer

A recent report demonstrates that the E2 protein of the hepatitis C virus (HCV) can activate ATF-2 through the MAPK/ERK pathway, thus promoting cell proliferation in human hepatoma Huh-7 cells.[98] In addition, a study in a large number of human lung cancer cell lines has revealed *ATF-2* genetic variants in 10.6% of these cell lines, which correspond to various histological types. Three of the five variants are genetic polymorphisms, while the remaining two are possibly somatic mutations.[99] These studies provide hints of positive *ATF-2* association with these types of cancer, but its exact role remains to be elucidated.

Melanoma

Melanoma is a special skin cancer type caused by gradual transformation of melanocytes into melanoma cells, mainly due to exposure to UV irradiation. Late-stage melanoma cells MeWo are highly metastatic and UV radiation-resistant. Upon UV irradiation, ATF-2 is overexpressed in these cells and binds to UV-Responsive Element (URE: TGACAACA) located within promoter sequences of stress-responsive genes. A dominant negative mutant form of ATF-2, which lacks the transactivation domain, decreases UV-resistance, while this feature is restored by subsequent transfection of cells with wild-type ATF-2. Similar results are observed in the early-stage melanoma cells WM3211 although, in this case, the metastatic potential of melanoma cells remains unaffected.[4] This cancer characteristic could be mediated by other CREB proteins such as CREB, since a dominant negative CREB mutant inhibits tumour growth and metastasis in MeWo cells.[100] Subcellular localization of ATF-2 has been proposed as a useful prognostic marker, since immunohistochemical staining in melanoma specimens from 544 patients has revealed that active nuclear ATF-2 is correlated with poor survival, whereas inactive cytoplasmic ATF-2 is correlated with greater life expectancy in these patients.[101]

Mouse models in understanding ATF-2 mode of action in carcinogenesis

Mouse study models provide invaluable in vivo evidence that *ATF-2* is closely related to skin cancer development. A recent study showed that, in mouse skin, epidermal cells, JB6 C141, TPA (12-0-tetradecanoylphorbol-13-acetate)-induced cell transformation involves ATF-2, c-Jun and a novel characterized Serine/Threonine protein kinase, MLTK-α. MLTK-α belongs to the MLTK (MLK-like MAP triple kinases, also referred as Zipper sterile-α-motif kinases) family of mixed-lineage kinases, which activate JNK, p38 and ERK kinases. TPA promotes ATF-2 and c-Jun phosphorylation through the MLTK-α-JNK/p38 axis. Injections with MLTK-α-overexpressing JB6 C141 cells in athymic nude mice resulted in tumour formation, while injections with si-MLTK-α-transfected cells failed to cause tumour formation.[102]

In addition, ATF-2 is thoroughly investigated in the multistage mouse skin carcinogenesis system, developed after chemical treatment of mouse epidermis with DMBA (7,12-dimethylbenz(a)anthracene) and TPA. This model comprises a series of cell lines isolated from different stages of mouse skin tumour progression, which represent distinct carcinogenesis stages. It includes immortalized, non-tumourigenic keratinocyte cell lines (C5N), benign papilloma cell lines (P1, P6) as well as squamous carcinoma cell lines (B9), which give rise to well-differentiated tumours upon injection into nude mice. It also includes highly anaplastic, invasive spindle cell lines (A5, CarB) which promote aggressive tumour growth and metastasis in vivo (reviewed in Ref. 103). In this model, we have observed a gradual increase in the levels of AP-1 components c-Jun, Fra-1, Fra-2 and ATF-2, during transition from immortalized to invasive cell lines, while JunD levels remained stable and JunB levels were only elevated in squamous carcinoma cell line. Elevated JNK activity and remarkably high levels of ATF-2 were detected in B9, A5 and CarB cell lines, followed by increased binding and transcriptional activity of ATF-2 to CRE and Jun2TRE elements.[8] We further overexpressed a dominant negative form of ATF-2, which cannot be phosphorylated due to 69 and 71 Thr/Ala substitutions, but can be dimerized through its intact bZIP domain, in the invasive spindle cell lines and assayed their behavior in vitro and in vivo. The stable transfectants demonstrated altered composition and decreased activity of the AP-1 complex, followed by significant downregulation of *cyclin D1, cyclin A* and *ATF-3* gene targets. The dominant negative ATF-2 transfected mice presented much smaller tumours with an epithelial-like appearance, in comparison to control mice, which were injected with parental or empty vector-transfected cells. Conclusively, aggressive characteristics were suppressed in the dnATF-2 transfected mice, indicating that ATF-2 overexpression is required for tumour growth and progression in mouse skin oncogenesis.[9] The fact that ATF-2 levels are significantly elevated in more-aggressive mouse skin carcinogenesis stages, as well as the observation that dnATF-2 altered the oncogenic potential of these cells indicates that, among all bZIP proteins, ATF-2 has the key role in the advanced stages of this process.[9]

Collectively, these results demonstrate a proliferative role for ATF-2 in skin cancer progression, in response to JNK or/and p38 activation. c-Jun seems to be an essential co-operator for ATF-2 during this process.

A theoretical basis for inhibition of ATF-2-promoted proliferation in cancer

Based on the findings in mouse study models, we can propose a model of ATF-2-promoted proliferation in skin carcinogenesis upon chemical treatment (see Fig. 5A), as well as a theoretical basis for its inhibition (see Fig. 5B). The initiating step in this process seems to be DMBA-promoted mutations in the *ras* gene. Constant activation of the Ras pathway, results in permanent phosphorylation of downstream kinases p38, JNK and ERK. c-Jun and ATF-2 are subsequently phosphorylated and form homodimers or/and heterodimers that bind to Jun2TRE element, enhancing *c-jun* transcription, as well as to CRE elements, enhancing transcription of genes that enable cell cycle progression and cell proliferation (e.g. *cyclin A, cyclin D1, ATF-3*). As carcinogenesis proceeds, overexpression of these crucial genes contributes to acquisition of more-aggressive cancer characteristics (see Fig. 5A).

Upon dnATF-2 transfection, antagonism between wild-type ATF-2 and mutant ATF-2 takes place at two distinct levels: First, mutant ATF-2 abrogates the wild-type ATF-2 phosphorylation by occupying the MAP kinases' active site. Second, mutant ATF-2 proteins form inactive dnATF-2–c-Jun or/and dnATF-2–ATF-2 heterodimers, which bind to gene target promoters, but they are incapable of activating them. Therefore, transcription of *cyclin A*, *cyclin D*, *ATF-3* as well as enhancement of *c-jun* is suppressed, leading to cell-cycle arrest and abolishment of cell proliferation (see Fig. 5B).

Thus, the use of dnATF-2 in targeting against ATF-2 could block proliferation of cancer cells in skin tumours. This approach might possibly be applicable to a range of cancer types, such as melanoma,[4] breast cancer,[40] or even other types where ATF-2 presents a well-documented proliferative role; however, in vivo models are essential in order to test if this generalization holds true.

Future perspectives

On the one hand, significant in vitro and in situ experiments demonstrate mainly a proliferative role for *ATF-2* in several types of cancer, although studies in other cancer types, e.g. leukemia, argue against this role. On the other hand, robust in vivo evidence from mouse study models positively correlates ATF-2 with cancer progression. The fact that ATF-2 function is affected by selective dimerizations with other bZIP proteins may explain the controversial *ATF-2* effects, since different

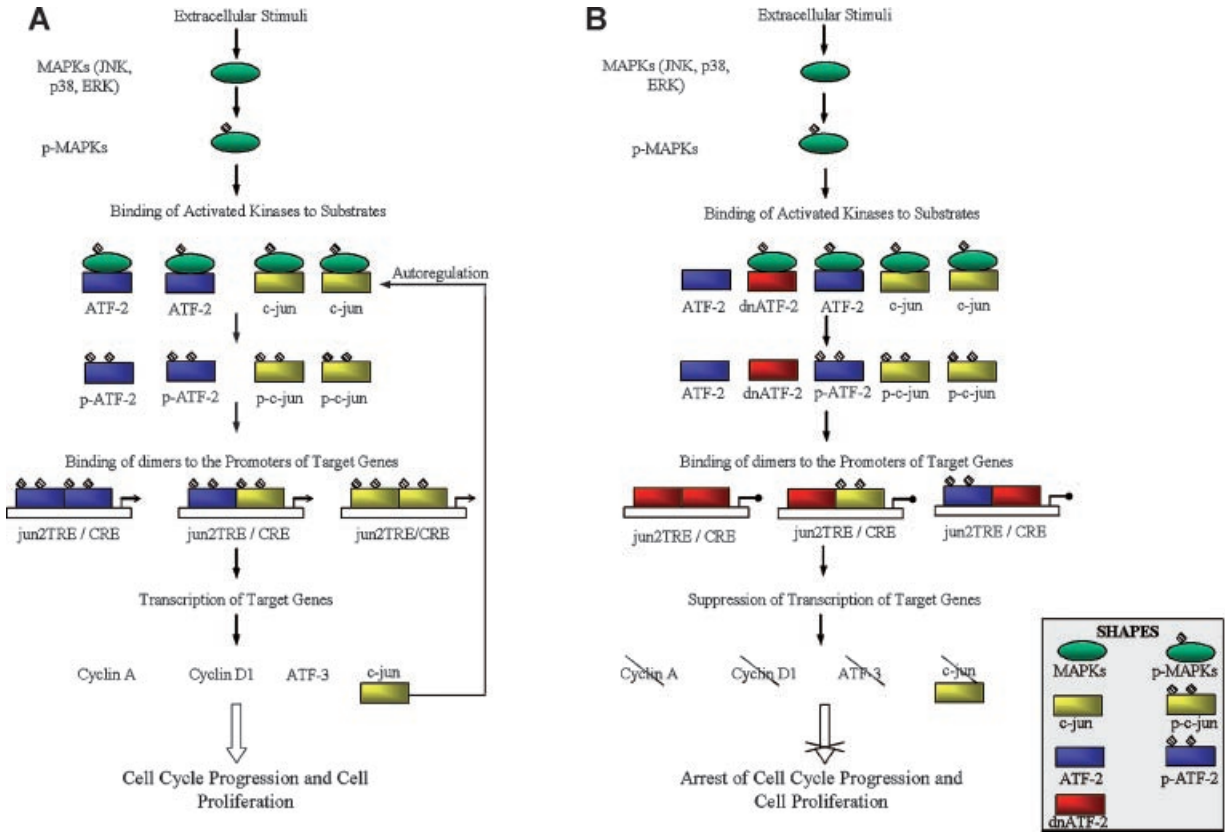

Figure 5. Model of ATF-2 action stimulated by oncogenic Ras and its inhibition. **A:** Downstream kinases p38, JNK and ERK phosphorylate c-Jun and ATF-2. c-Jun–ATF-2 heterodimers activate cyclin A, cyclin D1 and ATF-3, which contribute to acquisition of more aggressive cancer characteristics. **B:**. Effect of dominant negative ATF-2 in mouse skin oncogenesis: dnATF-2 antagonizes wild-type ATF-2 for binding to the MAPKs' active site. Then, mutant ATF-2 forms inactive homodimers or heterodimers with c-Jun, thus suppressing transcription of gene targets and leading to arrest of cell-cycle progression and cell proliferation.

bZIP content in different tissues could result in either proliferative or anti-proliferative *ATF-2* effect during carcinogenesis. Perhaps, the most-fruitful approach in studying not only ATF-2, but also several other ATF/CREB proteins with a highly controversial role in oncogenesis would be in relation with its dimerizing partners.

An emerging bZIP family interplay in both transcriptional and posttranslational levels involves complex interactions of bZIP proteins based on regulation of transcription of bZIP proteins by other bZIP dimers, selective dimerizations as well as different binding affinities of bZIP dimers for responsive elements (see Fig. 4). These interactions might serve as a potential sophisticated, overall mechanism of differential gene activation, which enables cell homeostasis. The fact that ATF-2 participates in all these types of interaction implies a key role as a modifier of processes that are generally deregulated in cancer, i.e. differentiation, proliferation and apoptosis.

ATF-2 represents the last molecule in the Ras-activated p38/JNK cascade, which not only has the ability to select the final target genes according to its upstream signals, but also to dynamically affect the bZIP protein composition in the cell and to modify bZIP interactions. Thus, it could serve as a molecular target in cancer therapy, at least for the subset of cancers where its proliferative role is well documented. In Ras-promoted carcinogenesis, dominant negative ATF-2 could be used in order to block proliferation of cancer cells. Since dominant negative ATF-2 has been shown to efficiently inhibit cell proliferation in mouse skin cancer, as well as in human breast cancer and melanoma, it might prove to be a promising anticancer agent for these types of cancer. A recent in silico analysis providing evidence that *ATF-2* is likely to be targeted by microRNAs adds a fascinating perspective in development of therapeutic agents against *ATF-2*.[104] Blocking peptides of ATF-2[105] could also be considered as a source for pharmacomimetic drug design. Combination of ATF-2 blocking together with its upstream kinases' inhibition could dramatically increase the degree of cell cycle arrest and apoptosis. Furthermore, anticancer

therapy might generally be benefit not only by ATF-2 blocking, but also by favoring synthesis of those bZIP proteins that form inert dimers with ATF-2, c-jun or/ and other bZIP proteins with a proved proliferative role, thus altering bZIP protein interactions towards an antiproliferative or apoptotic cellular bZIP status. Future studies towards the directions mentioned above may facilitate the planning of successful treatment protocols against the disease.

References

1. Hanahan D, Weinberg RA. 2000. The hallmarks of cancer. Cell 100:57–70.
2. Van Dyke T, Jacks T. 2002. Cancer modeling in the modern era: progress and challenges. Cell 108:135–144.
3. Echlin DR, Tae HJ, Mitin N, Taparowsky EJ. 2000. B-ATF function as a negative regulator of AP-1 mediated transcription and blocks cellular transformation by Ras and Fos. Oncogene 19:1752–1763.
4. Ronai Z, Yang YM, Fuchs SY, Adler V, Sardana M, et al. 1998. ATF2 confers radiation resistance to human melanoma cells. Oncogene 16:523–531.
5. Hai T, Hartman MG. 2001. The molecular biology and nomenclature of the activating transcription factor/cAMP responsive element binding family of transcription factors: activating transcription factor proteins and homeostasis. Gene 273:1–11.
6. Ozawa K, Sudo T, Soeda E, Yoshida MC, Ishii S. 1991. Assignment of the human CREB2 (CRE-BP1) gene to 2q32. Genomics 10:1103–1104.
7. Reimold AM, Grusby MJ, Kosaras B, Fries JW, Mori R, et al. 1996. Chondrodysplasia and neurological abnormalities in ATF-2 deficient mice. Nature 379:262–265.
8. Zoumpourlis V, Papassava P, Linardopoulos S, Gillespie D, Balmain A, et al. 2000. High levels of phosphorylated c-Jun, Fra-1, Fra-2 and ATF-2 proteins correlate with malignant phenotypes in the multistage mouse skin carcinogenesis model. Oncogene 19:4011–4021.
9. Papassava P, Gorgoulis V, Papaevangeliou D, Vlahopoulos S, van Dam H, et al. 2004. Overexpression of Activated Transcription Factor 2 is required for tumour growth and progression in mouse skin tumours. Cancer Res 64:8573–8584.
10. Wang J, Cao Y, Steiner DF. 2003. Regulation of proglucagon transcription by activated transcription factor (ATF) 3 and a novel isoform, ATF3b, through the cAMP-response element/ATF site of the proglucagon gene promoter. J Biol Chem 278:32899–32904.
11. Haze K, Okada T, Yoshida H, Yanagi H, Yura T, et al. 2001. Identification of the G13 (cAMP-response-element-binding protein-related protein) gene product related to activating transcription factor 6 as a transcriptional activator of the mammalian unfolded protein response. Biochem J 355:19–28.
12. Pescini R, Kaszubska W, Whelan J, DeLamarter JF, Hooft van Huijsduijnen R. 1994. ATF-a0, a novel variant of the ATF/CREB transcription factor family, forms a dominant transcription inhibitor in ATF-a heterodimers. J Biol Chem 269:1159–1165.
13. Forgacs E, Gupta SK, Kerry JA, Semmes OJ. 2005. The bZIP transcription factor ATFx binds human T-cell leukemia virus type 1 (HTLV-1) Tax and represses HTLV-1 long terminal repeat-mediated transcription. J Virol 79:6932–6939.
14. Masquilier D, Foulkes NS, Mattei MG, Sassone-Corsi P. 1993. Human CREM gene: evolutionary conservation, chromosomal localization, and inducibility of the transcript. Cell Growth Differ 4:931–937.
15. Liu F, Thompson MA, Wagner S, Greenberg ME, Green MR. 1993. Activating transcription factor-1 can mediate Ca(2+)- and cAMP-inducible transcriptional activation. J Biol Chem 268:6714–6720.
16. Nomura N, Zu YL, Maekawa T, Tabata S, Akiyama T, et al. 1993. Isolation and characterization of a novel member of the gene family encoding the cAMP response element-binding protein CRE- BP1. J Biol Chem 268:4259–4263.
17. Gaire M, Chatton B, Kedinger C. 1990. Isolation and characterization of two novel, closely related ATF cDNA clones from HeLa cells. Nucleic Acids Res 18:3467–3473.
18. Tong WY, Nagano-Fujii M, Hidajat R, Deng L, Takigawa Y, et al. 2002. Physical interaction between hepatitis C virus NS4B protein and CREB-RP/ATF6beta. Biochem Biophys Res Commun 299:366–372.
19. Dorsey MJ, Tae HJ, Sollenberger KG, Mascarenhas NT, Johansen LM, et al. 1995. B-ATF: a novel human bZIP protein that associates with members of the AP-1 transcription factor family. Oncogene 11:2255–2265.
20. Landshulz WH, Johnson PF, McKnight SL. 1988. The leucine zipper: a hypothetical structure common to a new class of DNA binding proteins. Science 240:1759–1764.
21. Hai T, Liu F, Coukos WJ, Green MR. 1989. Transcription factor ATF cDNA clones: An extensive family of leucine zipper proteins able to selectively form DNA-binding heterodimers. Genes Dev 3:2083–2090.
22. Vinson CR, Hai T, Boyd SM. 1993. Dimerization specificity of the leucine zipper-containing bZIP motif on DNA binding: prediction and rational design. Genes Dev 7:1047–1058.
23. Lin YS, Green MR. 1988. Interaction of a common transcription factor, ATF, with regulatory elements in both E1a-and cyclic AMP-inducible promoters. Proc Natl Acad Sci USA 85:3396–3400.
24. Leslie MC, Bar-Eli M. 2005. Regulation of gene expression in melanoma: new approaches for treatment. J Biol Chem 94:25–38.
25. Ghoneim C, Soula-Rothut M, Blanchevoye C, Martini L, Antonicelli F, et al. 2007. Activating transcription factor-1 mediated hepatocyte growth factor-induced down-regulation of thrombospondin-1 expression leads to thyroid cancer cell invasion. J Biol Chem 282:15490–15497.
26. Igarashi T, Izumi H, Uchiumi T, Nishio K, Arao T, et al. 2007. Clock and ATF4 transcription system regulates drug resistance in human cancer cell lines. Oncogene 26:4749–4760.
27. Linnerth NM, Baldwin M, Campbell C, Brown M, McGowan H, et al. 2005. IGF-II induces CREB phosphorylation and cell survival in human lung cancer cells. Oncogene 24:7310–7319.
28. Arai M, Kondoh N, Imazeki N, Hada A, Hatsutse K, et al. 2006. Transformation-associated gene regulation by ATF6alpha during hepatocarcinogenesis. FEBS Lett 580:184–190.
29. Persengiev SP, Devireddy LR, Green MR. 2002. Inhibition of apoptosis by ATFx: A novel role for a member of the ATF/CREB family of mammalian bZIP transcription factors. Genes Dev 16:1806–1814.
30. Bandyopadhyay S, Wang Y, Zhan R, Pai SK, Watabe M, et al. 2006. The tumour metastasis suppressor gene Drg-1 down-regulates the expression of activating transcription factor 3 in prostate cancer. Cancer Res 66:11983–11990.
31. Syed V, Macherjee K, Lyons-Weiler J, Lau KM, Mashima T, et al. 2005. Identification of ATF-3, caveolin-1, DLC-1, and NM23-H2 as putative antitumourigenic, progesterone-regulated genes for ovarian cancer cells by gene profiling. Oncogene 24:1774–1787.
32. Ricote M, Garcia-Tunon I, Bethencourt F, Fraile B, Onsurbe P, et al. 2006. The p38 transduction pathway in prostatic neoplasia. J Pathol 208:401–407.
33. Maekawa T, Shinagawa T, Sano Y, Sakuma T, Nomura S, et al. 2006. Reduced levels of ATF-2 predispose mice to mammary tumours. Mol Cell Biol 27:1730–1744.
34. Nagase T, Sudo T, Maekawa T, Yoshimura T, Fujisawa J, et al. 1990. Promoter region of the human CRE-BP1 gene encoding the transcriptional regulator binding to the cyclic AMP response element. J Biol Chem 265:17300–17306.
35. Li XY, Green MR. 1996. Intramolecular inhibition of activating transcription factor-2 function by its DNA-binding domain. Genes Dev 10:517–527.
36. Gupta S, Campbell D, Derijard B, Davis RJ. 1995. Transcription factor ATF2 regulation by the JNK signal transduction pathway. Science 267:389–393.
37. Livingstone C, Patel G, Jones N. 1995. ATF-2 contains a phosphorylation-dependent transcriptional activation domain. EMBO J 14:1785–1797.

38. Raingeaud J, Gupta S, Rogers JS, Dickens M, Han J, et al. 1995. Pro-inflammatory cytokines and environmental stress cause p38 mitogen activated kinase activation by dual phosphorylation on tyrosine and threonine. J Biol Chem 270:7420–7426.
39. Beier F, Taylor AC, LuValle P. 2000. Activating transcription factor 2 is necessary for maximal activity and serum induction of the cyclin A promoter in chondrocytes. J Biol Chem 275:12948–12953.
40. Hayakawa J, Depatie C, Ohmichi M, Mercola D. 2003. The activation of c-Jun NH2-terminal kinase (JNK) by DNA-damaging agents serves to promote drug resistance via activating transcription factor 2 (ATF2)-dependent enhanced DNA repair. J Biol Chem 278:20582–20592.
41. Hayakawa J, Mittal S, Wang Y, Korkmaz KS, Adamson E, et al. 2004. Identification of promoters bound by c-Jun/ATF2 during rapid large scale gene activation following genotoxic stress. Mol Cell 16:521–535.
42. Vlahopoulos SA, Zoumpourlis VC. 2004. JNK: a key modulator of intracellular signaling. Biochemistry (Moscow) 69:844–854.
43. Ouwens DM, de Ruiter ND, van der Zon G, Carter AP, Schouten J, et al. 2002. Growth factors can activate ATF2 via a two-step mechanism: phosphorylation of Thr71 through the Ras–MEK-ERK pathway and of Thr69 through RalGDS-Src–p38. EMBO J 21:3782–3793.
44. Foulkes NS, Laoide BM, Schlotter F, Sassone-Corsi P. 1991. Transcriptional antagonist cAMP-responsive element modulator (CREM) down-regulates c-fos cAMP-induced expression. Proc Natl Acad Sci USA 88:5448–5452.
45. Aronheim A, Zandi E, Hennemann H, Elledge SJ, Karin M. 1997. Isolation of an AP-1 repressor by a novel method for detecting protein-protein interactions. Mol Cell Biol 17:3094–3102.
46. Wang Y, Shen J, Arenzana N, Tirasophon W, Kaufman RJ, et al. 2000. Activation of ATF6 and an ATF6 DNA binding site by the endoplasmic reticulum stress response. J Biol Chem 275:27013–27020.
47. White JH, McIlhinney RA, Wise A, Ciruela F, Chan WY, et al. 2000. The GABAB receptor interacts directly with the related transcription factors CREB2 and ATFx. Proc Natl Acad Sci USA 97:13967–13972.
48. Jin C, Ugai H, Song J, Murata T, Nili F, et al. 2001. Identification of mouse Jun dimerization protein 2 as a novel repressor of ATF-2. FEBS Lett 489:34–41.
49. Thiel G, Al Sarraj J, Vinson C, Stefano L, Bach K. 2005. Role of basic region leucine zipper transcription factors cyclic AMP response element binding protein (CREB), CREB2, activating transcription factor 2 and CAAT/enhancer binding protein alpha in cyclic AMP response element-mediated transcription. J Neurochem 92:321–336.
50. Acharya A, Rishi V, Moll J, Vinson C. 2006. Experimental identification of homodimerizing B-ZIP families in Homo sapiens. J Struct Biol 155:130–139.
51. Hsu JC, Laz T, Mohn KL, Taub R. 1991. Identification of LRF-1, a leucine-zipper protein that is rapidly and highly induced in regenerating liver. Proc Natl Acad Sci USA 88:3511–3515.
52. Herdegen T, Leah JD. 1998. Inducible and constitutive transcription factors in the mammalian nervous system: control of gene expression by Jun, Fos and Krox, and CREB/ATF proteins. Brain Res Brain Res Rev 28:370–490.
53. Hai T, Curran T. 1991. Cross-family dimerization of transcription factors Fos/Jun and ATF/CREB alters DNA binding specificity. Proc Natl Acad Sci USA 88:3720–3724.
54. Masquilier D, Sassone-Corsi P. 1992. Transcriptional cross-talk: nuclear factors CREM and CREB bind to AP-1 sites and inhibit activation by Jun. J Biol Chem 267:22460–22466.
55. Kerppola TK, Curran T. 1993. Selective DNA bending by a variety of bZIP proteins. Mol Cell Biol 13:5479–5489.
56. Chu HM, Tan Y, Kobierski LA, Balsam LB, Comb MJ. 1994. Activating transcription factor-3 stimulates 3′,5′-cyclic adenosine monophosphate-dependent gene expression. Mol Endocrinol 8:59–68.
57. Chatton B, Bocco JL, Goetz J, Gaire M, Lutz Y, et al. 1994. Jun and Fos heterodimerize with ATFa, a member of the ATF/CREB family and modulate its transcriptional activity. Oncogene 9:375–385.
58. Shimizu M, Nomura Y, Suzuki H, Ichikawa E, Takauchi A, et al. 1998. Activation of the rat cyclin A promoter by ATF2 and Jun family members and its suppression by A TF4. Exp Cell Res 239:93–103.
59. Castellanos Mdel C, López-Giral S, López-Cabrera M, de Landázuri MO. 2002. Multiple cis-acting elements regulate the expression of the early T cell activation antigen CD69. Eur J Immunol 32:3108–3117.
60. Benbrook DM, Jones NC. 1990. Heterodimer formation between CREB and JUN proteins. Oncogene 5:295–302.
61. Song H, Ki SH, Kim SG, Moon A. 2006. Activating transcription factor 2 mediates matrix metalloproteinase-2 transcriptional activation induced by p38 in breast epithelial cells. Cancer Res 66:10487–10496.
62. De Cesare D, Vallone D, Caracciolo A, Sassone-Corsi P, Nerlov C, et al. 1995. Heterodimerization of c-Jun with ATF-2 and c-Fos is required for positive and negative regulation of the human urokinase enhancer. Oncogene 11:365–376.
63. Penix LA, Sweetser M, Weaver WM, Hoeffler JP, Kerppola TK, et al. 1996. The proximal regulatory element of the interferon-γ promoter mediates selective expression in T cells. J Biol Chem 271:31964–31972.
64. Gong P, Stewart D, Hu B, Vinson C, Alam J. 2002. Multiple basic-leucine zipper proteins regulate induction of the mouse heme oxygenase-1 gene by arsenite. Arch Biochem Biophys 405:265–274.
65. Beier F, Lee RJ, Taylor AC, Pestell RG, LuValle P. 1999. Identification of the cyclin D1 gene as a target of activating transcription factor 2 in chondrocytes. Proc Natl Acad Sci USA 96:1433–1438.
66. Recio JA, Merlino G. 2002. Hepatocyte growth factor/scatter factor activates proliferation in melanoma cells through p38 MAPK, ATF-2 and cyclin D1. Oncogene 21:1000–1008.
67. Gavrilov D, Kenzior O, Evans M, Calaluce R, Folk WR. 2001. Expression of urokinase plasminogen activator and receptor in conjunction with the ets family and AP-1 complex transcription factors in high grade prostate cancers. Eur J Cancer 37:1033–1040.
68. Maekawa T, Bernier F, Sato M, Nomura S, Singh M, et al. 1999. Mouse ATF-2 null mutants display features of a severe type of meconium aspiration syndrome. J Biol Chem 274:17813–17819.
69. Bates RC, DeLeo MJ 3rd, Mercurio AM. 2004. The epithelial-mesenchymal transition of colon carcinoma involves expression of IL-8 and CXCR-1-mediated chemotaxis. Exp Cell Res 299:315–324.
70. Eliopoulos AG, Gallagher NJ, Blake SM, Dawson CW, Young LS. 1999. Activation of the p38 mitogen-activated protein kinase pathway by Epstein-Barr virus-encoded latent membrane protein 1 coregulates interleukin-6 and interleukin-8 production. J Biol Chem 274:16085–16096.
71. Laferriere J, Houle F, Taher MM, Valerie K, Huot J. 2001. Transendothelial migration of colon carcinoma cells requires expression of E-selectin by endothelial cells and activation of stress-activated protein kinase-2 (SAPK2/p38) in the tumour cells. J Biol Chem 276:33762–33772.
72. Read MA, Whitley MZ, Gupta S, Pierce JW, Best J, et al. 1997. Tumour necrosis factor alpha-induced E-selectin expression is activated by the nuclear factor-kappaB and c-JUN N-terminal kinase/p38 mitogen-activated protein kinase pathways. J Biol Chem 272:2753–2761.
73. Ma Q, Li X, Vale-Cruz D, Brown ML, Beier F, et al. 2007. Activating transcription factor 2 controls Bcl-2 promoter activity in growth plate chondrocytes. J Cell Biochem 101:477–487.
74. Mechta-Grigoriou F, Gerald D, Yaniv M. 2001. The mammalian Jun proteins: redundancy and specificity. Oncogene 20:2378–2389.
75. Ivashkiv LB, Liou HC, Kara CJ, Lamph WW, Verma IM, et al. 1990. mXBP/CRE-BP2 and c-Jun form a complex which binds to the cyclic AMP, but not to the 12-O-tetradecanoylphorbol-13-acetate, response element. Mol Cell Biol 10:1609–1621.
76. van Dam H, Duyndam M, Rottier R, Bosch A, de Vries-Smits L, et al. 1993. Heterodimer formation of c-Jun and ATF-2 is responsible for induction of cJun by the 243 amino acid adenovirus E1A protein. EMBO J 12:479–487.
77. van Dam H, Wilhelm D, Herr I, Steffen A, Herrlich P, et al. 1995. ATF-2 is preferentially activated by stress-activated protein kinases to mediate c-jun induction in response to genotoxic agents. EMBO J 14:1798–1811.
78. van Dam H, Castellazzi M. 2001. Distinct roles of Jun:Fos and Jun:ATF dimmers in oncogenesis. Oncogene 20:2453–2464.

79. Liang G, Wolfgang CD, Chen CB, Chen TH, Hai T. 1996. ATF3 gene. Genomic organization, promoter, and regulation. J Biol Chem 271: 1695–1701.
80. Lu D, Wolfgang CD, Hai T. 2006. Activating transcription factor 3, a stress-inducible gene, suppresses Ras-stimulated tumourigenesis. J Biol Chem 281:10473–10481.
81. Yan C, Lu D, Hai T, Boyd DD. 2005. Activating transcription factor 3, a stress sensor, activates p53 by blocking its ubiquitination. EMBO J 24: 2425–2435.
82. Allan AL, Albanese C, Pestell RG, LaMarre J. 2001. Activating transcription factor 3 induces DNA synthesis and expression of cyclin d1 in Hepatocytes J Biol Chem. 276:27272–27280.
83. Steinmuller L, Thiel G. 2003. Regulation of gene transcription by a constitutively active mutant of activating transcription factor 2 (ATF2). Biol Chem 384:667–672.
84. Steinmuller L, Cibelli G, Moll JR, Vinson C, Thiel G. 2001. Regulation and composition of activator protein 1 (AP-1) transcription factors controlling collagenase and c-Jun promoter activities. Bioch J 360: 599–607.
85. Hess J, Angel P, Schrorpp-Kistner M. 2004. AP-1 subunits: quarell and harmony among siblings J Cell Sci. 117:5965–5973.
86. Wang X, Studzinski GP. 2006. The requirement for and changing composition of the activating protein-1 transcription factor during differentiation of human leukemia HL60 cells induced by 1,25-Dihydroxyvitamin D3. Cancer Res 66:4402–4409.
87. van Dam H, Huguier S, Kooistra K, Baguet J, Vial E, et al. 1998. Autocrine growth and anchorage independence: two complementing Jun-controlled genetic programs of cellular transformation. Genes Dev 12: 1227–1239.
88. Hadzopoulou-Cladaras M, Lavrentiadou SN, Zannis VI, Kardassis D. 1998. Transactivation of the ApoCIII promoter by ATF-2 and repression by members of the Jun family. Biochemistry 37:14078–14087.
89. Pyrzynska B, Mosieniak G, Kaminska B. 2000. Changes of the trans-activating potential of AP-1 transcription factor during cyclosporin A-induced apoptosis of glioma cells are mediated by phosphorylation and alterations of AP-1 composition. J Neurochem 74:42–51.
90. Yemelyanov A, Czwornog J, Chebotaev D, Karseladze A, Kulevitch E, et al. 2007. Tumour suppressor activity of glucocorticoid receptor in the prostate. Oncogene 26:1885–1896.
91. Lewis JS, Vijayanathan V, Thomas TJ, Albanese C, Gallo MA, et al. 2005. Activation of cyclin D1 by estradiol and spermine in MCF-7 breast cancer cells: a mechanism involving the p38 MAP kinase and phosphorylation of ATF-2. Oncol Res 15:113–128.
92. Xue L, Firestone GL, Bjeldanes LF. 2005. DIM stimulates IFNgamma gene expression in human breast cancer cells via the specific activation of JNK and p38 pathways. Oncogene 24:2343–2353.
93. Wang Q, Salman H, Danilenko M, Studzinski GP. 2005. Cooperation between antioxidants and 1,25-dihydroxyvitamin D3 in induction of leukemia HL60 cell differentiation through the JNK/AP-1/Egr-1 pathway. J Cell Physiol 204:964–974.
94. Wu J, Suzuki H, Akhand AA, Zhou YW, Hossain K, et al. 2002. Modes of activation of mitogen-activated protein kinases and their roles in cepharanthine-induced apoptosis in leukaemia cells. Cell Signal 14: 509–515.
95. Leppa S, Eriksson M, Saffrich R, Ansorge W, Bohmann D. 2001. Complex functions of AP-1 transcription factors in differentiation and survival of PC12 cells. Mol Cell Biol 21:4369–4378.
96. Tindberg N, Porsmyr-Palmertz M, Simi A. 2000. Contribution of MAP kinase pathways to the activation of ATF-2 in human neuroblastoma cells. Neurochem Res 25:527–531.
97. García-Silva S, Aranda A. 2004. The thyroid hormone receptor is a suppressor of *ras*-mediated transcription, proliferation, and transformation. Mol Cell Biol 24:7514–7523.
98. Zhao LJ, Wang L, Ren H, Cao J, Li L, et al. 2005. Hepatitis C virus E2 protein promotes human hepatoma cell proliferation through the MAPK/ERK signaling pathway via cellular receptors. Exp Cell Res 305:23–32.
99. Woo IS, Kohno T, Inoue K, Ishii S, Yokota J. 2002. Infrequent mutations of the activating transcription factor-2 gene in human lung cancer, neuroblastoma and breast cancer. Int J Oncol 20:527–531.
100. Xie S, Price JE, Luca M, Jean D, Ronai Z, et al. 1997. Dominant-negative CREB inhibits tumour growth and metastasis of human melanoma cells. Oncogene 15:2069–2075.
101. Berger AJ, Kluger HM, Li N, Kielhorn E, Halaban R, et al. 2003. Subcellular localization of activating transcription factor 2 in melanoma specimens predicts patient survival. Cancer Res 63:8103–8107.
102. Cho Y, Bode AM, Mizuno H, Choi BY, Choi HS, et al. 2004. A novel role for mixed-lineage kinase-like mitogen-activated protein triple kinase α in neoplastic cell transformation and tumour development. Cancer Res 64:3855–3864.
103. Zoumpourlis V, Solakidi S, Papathoma A, Papaevangeliou D. 2003. Alterations in signal transduction pathways implicated in tumour progression during multistage mouse skin carcinogenesis. Carcinogenesis 24:1159–1165.
104. Hansen T, Olsen L, Lindow M, Jakobsen KD, Ullum H, et al. 2007. Brain expressed microRNAs implicated in schizophrenia etiology. PLoS ONE 2:e873.
105. Bhoumik A, Ivanov V, Ronai Z. 2001. Activating transcription factor 2-derived peptides alter resistance of human tumour cell lines to ultraviolet irradiation and chemical treatment. Clin Cancer Res 7:331–342.