

# The Activator Protein-1 Transcription Factor in Respiratory Epithelium Carcinogenesis

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## Abstract

**Respiratory epithelium cancers are the leading cause of cancer-related death worldwide. The multistep natural history of carcinogenesis can be considered as a gradual accumulation of genetic and epigenetic aberrations, resulting in the deregulation of cellular homeostasis. Growing evidence suggests that cross-talk between membrane and nuclear receptor signaling pathways along with the activator protein-1 (AP-1) cascade and its cofactor network represent a pivotal molecular circuitry participating directly or indirectly in respiratory epithelium carcinogenesis. The crucial role of AP-1 transcription factor renders it an appealing target of future nuclear-directed anticancer therapeutic and chemoprevention approaches. In the present review, we will summarize the current knowledge regarding the implication of AP-1 proteins in respiratory epithelium carcinogenesis, highlight the ongoing research, and consider the future perspectives of their potential therapeutic interest. (Mol Cancer Res 2007;5(2):109–20)**

## Introduction

Elucidation of the molecular events underlying respiratory epithelium carcinogenesis still remains a challenge, although new therapeutic interventions are in advanced clinical testing or even in daily clinical practice based on mature preclinical findings (1, 2). Transcriptional regulation can significantly affect the course of growth-related diseases, such as cancer. Transcription of protein-coding genes is regulated by transcription factors, which are generally classified as basal and gene-specific transcription factors (3). Interactions of gene-specific transcription factors with other regulatory proteins and their mutual cross-talk may have enhancing or competitive effects on transcription rate (4, 5). The in-depth understanding of the mechanisms that govern gene expression during carcinogenesis seems to be a prerequisite for the design of drugs selectively affecting tumor-specific transcriptional patterns (6).

Much of the current anticancer research effort is focused on cell-surface receptors and their cognate upstream molecules because they provide the easiest route for drugs to affect cellular behavior, whereas agents acting at the level of transcription need to invade the nucleus. However, the therapeutic effect of surface receptor manipulation might be considered less than specific because their actions are modulated by complex interacting downstream signal transduction pathways. A pivotal transcription factor during respiratory epithelium carcinogenesis is activator protein-1 (AP-1). AP-1-regulated genes include important modulators of invasion and metastasis, proliferation, differentiation, and survival as well as genes associated with hypoxia and angiogenesis (7). Nuclear-directed therapeutic strategies might represent the next step in “targeted” anticancer treatment, and AP-1 is one of the most appealing candidate targets for these new generation agents.

## The Multistep Nature of Respiratory Epithelium Carcinogenesis

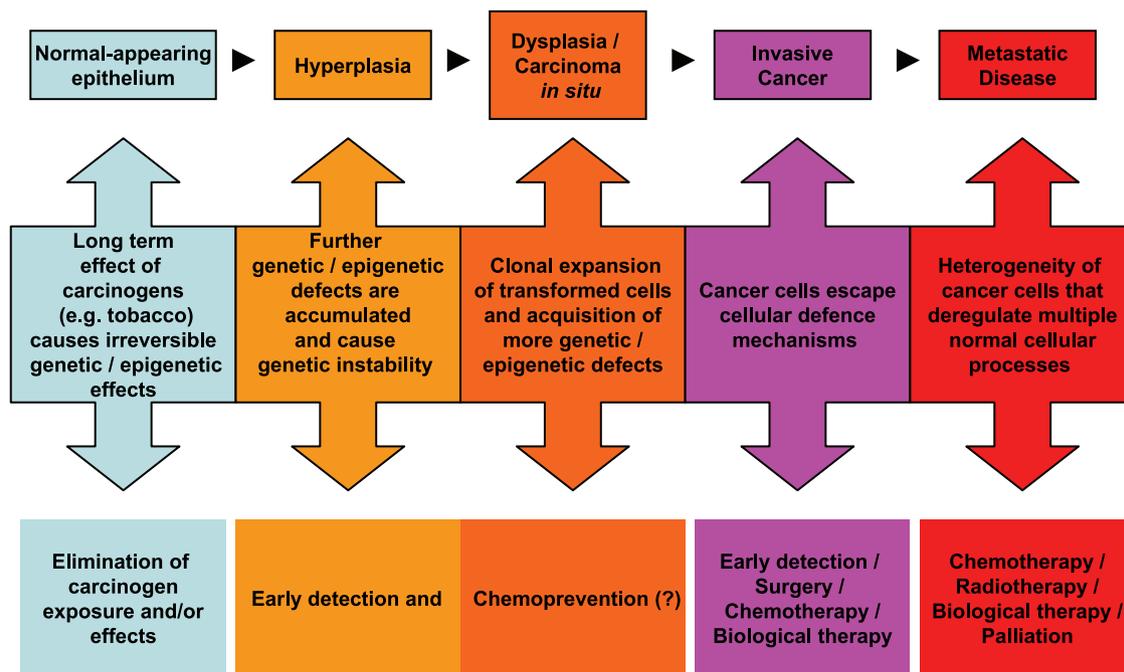
Carcinogenesis occurs through a series of phenotypic changes that parallel the accumulation of genetic events and epigenetic deviations (see Fig. 1). Field carcinogenesis is the multifocal development of premalignant and malignant lesions within the entire carcinogen-exposed area of an epithelial region, and this concept has been clearly established in respiratory epithelium (8). Many scientists suggest that more focus is needed in the evaluation and control of the initial steps of carcinogenesis, rather than trying to treat advanced stages.

Cancer chemoprevention represents the rational approach to this notion. It can be classified into primary in healthy individuals who have increased risk in developing cancer, secondary in individuals with already diagnosed premalignant lesions, and tertiary in patients who have been treated for a cancer and aims at preventing the development of a recurrence or a second primary tumor (9). Chemoprevention agents might be synthetic compounds or natural products. Many proposed mechanisms of action try to enlighten their anticancer effect, whereas the rapidly increasing knowledge in molecular oncology field has resulted in the extensive research of signal transduction pathways and their associated factors, with the primary goal being the identification of novel potential chemopreventive and/or therapeutic molecular targets (10).

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**FIGURE 1.** It has long been recognized that respiratory epithelium carcinogenesis proceeds through multiple distinct stages, each characterized by specific molecular and histologic alterations. Whereas there is a general trend to think about cancerization in a linear fashion, multiple stimuli from all carcinogenesis phases can operate simultaneously. Although the sequence of premalignant changes to invasive cancer has not been fully elucidated, it seems that hyperplastic and metaplastic histologic phases represent the decisive step in which crucial molecular defects deregulate cellular homeostasis, thereby triggering respiratory epithelium carcinogenesis. Whereas substantial advances have been made regarding early detection, prevention, and treatment of lung carcinomas, these are yet considered suboptimal.

## Genetics and Epigenetics in Respiratory Epithelium Carcinogenesis

Many factors contribute to respiratory epithelium carcinogenesis, including inherited and acquired genetic changes, chromosomal rearrangements, epigenetic phenomena, and chemical carcinogens (e.g., cigarette smoking; ref. 11). Long-term exposure to cigarette smoke induces oxidative stress, leading to activation of stress-triggered kinases and potentiation of various important transcription factors, such as AP-1 proteins (12, 13). Lung cancers arising in smokers have a different spectrum of molecular abnormalities than those seen in nonsmokers, suggesting differences in molecular etiology, pathogenesis, and possibly prognosis (14). For example, *K-ras* mutations occur predominantly in non-small cell lung cancers (NSCLC), are strongly correlated with smoking history, seem to be more common in women than men, and have been associated with poor prognosis (15, 16). Epidermal growth factor receptor (EGFR) is a transmembrane glycoprotein that is expressed in the majority of NSCLCs. Binding of ligands to its extracellular domain results in tyrosine kinase activation, with downstream effects including cell proliferation, differentiation, migration, motility, resistance to apoptosis, enhanced survival, and gene transcription (1). Several small molecules have been synthesized to inhibit the tyrosine kinase domain of EGFR (tyrosine kinase inhibitors) and produced clinically significant results (1). EGFR somatic mutations correlating with better clinical response to tyrosine kinase inhibitors have been identified (15), albeit such mutations have only been detected in the tyrosine kinase domain, are not always present in patients

responding to tyrosine kinase inhibitors, whereas recently inhibiting EGFR mutations have also been reported (17, 18). Accumulated preclinical and clinical evidence suggests that patients with *K-ras* mutant NSCLCs might represent a separate group of patients with inherent resistance to EGFR tyrosine kinase inhibitors (19-21). Overexpression of the *c-myc* oncogene is also frequently observed in NSCLCs but seems to be more prevalent in SCLC (22). Elevated expression of *HER-2/neu*, a member of the EGFR family, has also been observed in NSCLCs (23). Tumor-suppressor gene mutations (e.g., *p53* and *retinoblastoma*) are commonly found in lung carcinomas (24, 25) and seem to have a cardinal role in the early stages of carcinogenesis (26). Mutations in the *retinoblastoma* (*Rb*) gene occur in more than 90% of SCLCs, whereas only a small fraction of NSCLCs harbors such mutations (25).

Epigenetics (namely, DNA methylation and “histone code”) are also critical events during human lung tumorigenesis. The transfer of a methyl group to the C-5 position of cytosines, almost always in the context of CpG dinucleotides, requires two components: the DNA methyltransferases and the methyl CpG-binding proteins. DNA methylation is tightly connected to lung carcinogenesis due to deregulation of DNA methyltransferases, regional gene hypermethylation (tumor suppressor genes, genes involved in cell-cycle control, apoptosis, and drug sensitivity), or through global hypomethylation that enhances oncogene expression and predisposes to genomic instability (27). Several genes have been shown to be hypermethylated in lung cancer and in cases of

pre-malignant lesions and normal-appearing respiratory epithelium of high-risk individuals (28, 29). Various histone posttranslational modifications (i.e., methylation, acetylation, phosphorylation, and ubiquitination) can coordinately affect transcriptional control of genes by influencing the dynamic status of chromatin configuration (30). Epigenome alterations are relatively frequent, affect multiple genes, are potentially reversible, occur since the early stages of carcinogenesis, and are established through various enzymatic activities, which can be theoretically tackled.

### Molecular Anatomy of AP-1

AP-1 collectively describes a class of structurally and functionally related proteins that are characterized by a basic leucine-zipper region. It comprises members of the Jun protein family (c-Jun, JunB, and JunD) and Fos protein family. The Fos family of transcription factors includes c-Fos, FosB, Fos-related antigen-1 (Fra-1), and Fra-2 as well as smaller FosB splice variants FosB2 and DeltaFosB2 (31). Together, all these proteins form the group of AP-1 proteins that, after dimerization, bind to the so-called 12-*O*-tetradecanoylphorbol-13-acetate response elements in the promoter and enhancer regions of target genes. Additionally, some members of the ATF (ATFa, ATF-2, and ATF-3) and JDP (JDP-1 and JDP-2) subfamilies, which share structural similarities and form heterodimeric complexes with AP-1 proteins (predominantly Jun proteins), can bind to 12-*O*-tetradecanoylphorbol-13-acetate response element-like sequences (32, 33).

In contrast to Jun proteins, Fos family members are not able to form homodimers but heterodimerize with Jun partners, giving rise to various trans-activating or trans-repressing complexes with different biochemical properties (34). *In vitro* studies have shown that Jun-Fos heterodimers are more stable and display stronger DNA-binding activity than Jun-Jun homodimers (35, 36). The expression and stability of Fos proteins might be crucial for the activity of AP-1-regulated genes (36).

Notably, individual Jun and Fos proteins have significantly different trans-activation domains (31). Although c-Jun, c-Fos, and FosB proteins harbor a NH<sub>2</sub>-terminal trans-activation domain, JunB, JunD, Fra-1, Fra-2, and FosB2 exhibit only weak trans-activation activity (37). Accordingly, these proteins are not transforming in rat fibroblasts, and an inhibitory function of these factors on AP-1 activity, by competing for binding to AP-1 sites or by forming inactive heterodimers, has been proposed (38). Yet, recent results suggest that in many tumors, these non-transforming Fos proteins, especially Fra-1 and Fra-2, might be involved in the progression of many tumor types (31, 39).

The activity of AP-1 proteins can be modulated in a multilevel manner. They are usually induced in response to a broad spectrum of environmental cues, including cytokines, growth factors, stress signals, and oncogenic stimuli, which provoke the activation of various signal transduction pathways to transmit the signal from the extracellular milieu to the nucleus (40). Regulation can be achieved through changes in transcription of genes encoding AP-1 subunits, control of the stability of their mRNAs, posttranslational modifications and turnover of preexisting or newly synthesized AP-1 components, as well as via specific interactions of AP-1 proteins with other transcription factors and cofactors. Posttranslational

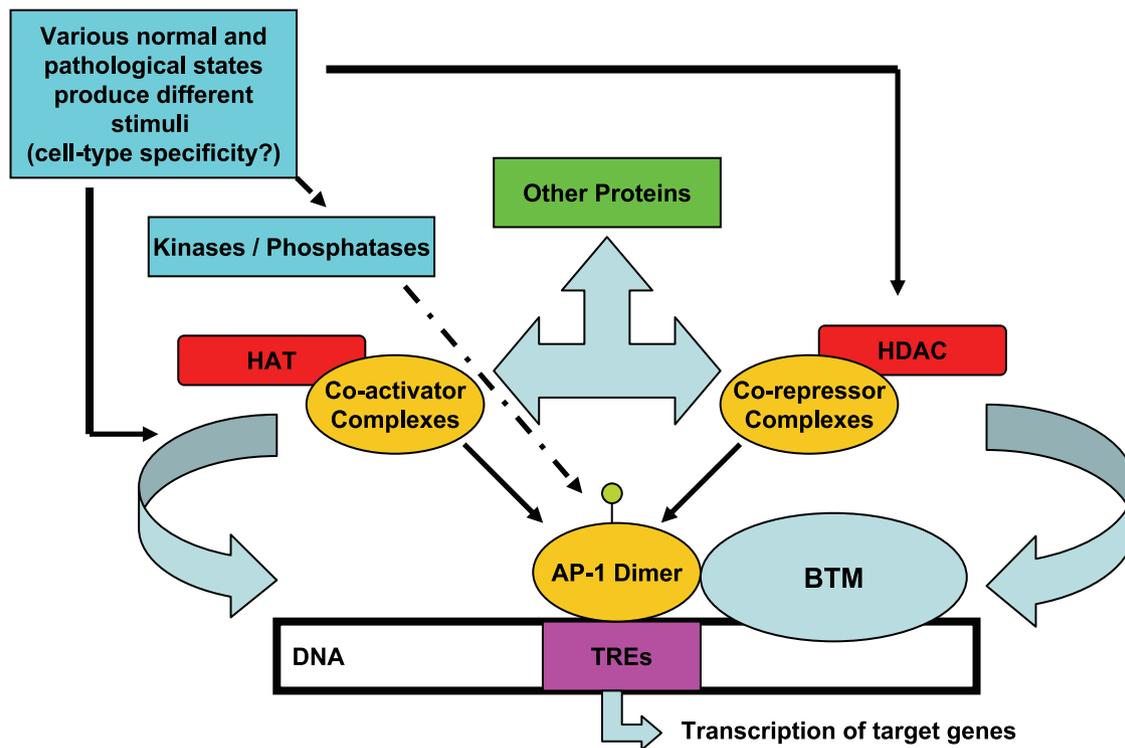
control mainly refers to phosphorylation by different kinases that influence DNA-binding activity, trans-activation potential, and protein stability (41, 42). The most extensively studied kinases are the Jun NH<sub>2</sub>-terminal kinases (JNK), which are members of the mitogen-activated protein kinase (MAPK) superfamily (43). Activated by MAPK cascade, JNKs translocate to the nucleus, whereby they phosphorylate Jun within its NH<sub>2</sub>-terminal trans-activation domain and thus elicit its trans-activation potential (44). JNKs also phosphorylate and activate JunD and ATF-2 (45, 46). By contrast, the kinases that regulate the activity of Fos proteins are not yet well characterized, although a plethora of candidates have been suggested (47-49).

Given the complexity and selectivity of AP-1 proteins action and modulation, it has been postulated that one AP-1-regulated gene might be preferentially induced by Jun-Fos dimers, whereas another gene is mainly stimulated by other dimeric species (50). Experimental data have also shown that single characteristics of the transformed phenotype (anchorage independence, serum-independent growth, and others) are triggered by specific Jun-Fos protein dimers (51). Generally, AP-1 proteins have both overlapping and unique roles and function in a tissue- and/or cell-specific fashion (52). Therefore, the measurement of AP-1 activity employing artificial AP-1-regulated promoter constructs, which was done in many early studies on cancer cells, is not very informative because this activity does not reflect the biological behavior of cancer cells. More recent studies have included the analysis of expression and/or activity of all Jun and Fos family members (53, 54). Using this approach, it was shown in several experimental systems that malignant transformation and progression is accompanied by a cell type-specific shift in AP-1 dimer composition (55).

### The AP-1 Cofactor Network

Regulation of gene expression at the transcriptional level is required for many cellular events and for the proper development of an organism. The essential nature of such control is exemplified by the plethora of proteins devoted to regulation of transcription by RNA polymerase II. However, the fundamental role of the transcription cofactors (coactivators and corepressors) has been only recently appreciated (refs. 56, 57; see Fig. 2). A large number of transcription cofactors are gradually identified. To date, the main target of their action has been their effect on modification of the histone components of chromatin. Histone acetylation is catalyzed in the nucleus by coactivators that share this enzymatic activity (histone acetyltransferase) and allows them to loosen the association of nucleosomes with the control region of a gene, and possibly also to reduce the interaction between individual nucleosomes, thereby enhancing transcription (58). One of the best-characterized coactivators with histone acetyltransferase activity is cyclic AMP response element-binding protein-binding protein (CBP)/p300. CBP is a transcriptional coactivator that acetylates lysine residues of histones and non-histone proteins, such as p53. It participates in basic cellular functions, including growth, differentiation, DNA repair, and apoptosis (59).

On the other hand, repressive cofactors use several distinct mechanisms, including competition with coactivator proteins



**FIGURE 2.** Transcription initiation by RNA polymerase II at eukaryotic protein-coding genes involves the cooperative assembly on the core promoter of multiple distinct proteins, including RNA polymerase II itself and basal transcription factors, to form a stable basal transcriptional machinery. This assembly is a major point of control by gene-specific transcription factors (activators and repressors) and is hindered by the packaging of promoter DNA into nucleosomes and higher order chromatin structures. Transcription cofactors (coactivators and corepressors) interact with gene-specific transcription factors and/or various components of the basal transcriptional machinery and are also essential for regulated transcription. BTM, basal transcriptional machinery; HAT, histone acetyltransferase; HDAC, histone deacetylase; TRE, 12-*O*-tetradecanoylphorbol-13-acetate response elements.

for DNA binding, sequestration of such activators, interaction with the basal transcriptional machinery, DNA methylation, and recruitment of complexes that bear histone deacetylase activity (60). Multi-protein complexes bring the histone deacetylases close to nucleosomes. The deacetylation of core histones allows their basic tails to bind strongly to DNA, stabilizing the nucleosome and inhibiting transcription (61). Such corepressors are silencing mediator of retinoic and thyroid hormone receptors (SMRT), nuclear receptor corepressor (N-CoR), metastatic tumor antigen 1 (MTA1), and others (62). MTA1 overexpression has been linked to the tumorigenesis and metastasis of respiratory epithelium carcinomas (63).

Concerning AP-1 proteins, it is noteworthy that they are capable of recruiting different transcription cofactors, depending on cell type or physiologic/pathologic context (ref. 64; see Fig. 2). Although AP-1 proteins are primarily associated with the regulation of cellular proliferation, it seems that one of their main features is their ability to cross-interact with various other crucial signal transduction pathways, thus affecting important cellular events. Based on this consideration, it might be feasible that different AP-1 dimers are formed in different tissue and/or cell type and in different stages of respiratory epithelium carcinogenesis. Moreover, in this regard, different protein complexes of transcriptional cofactors are recruited or formed and activate or suppress critical genes containing AP-1-dependent regulatory sites (65).

### The Role of Cross-talk of Signal Transduction Pathways and AP-1 in Respiratory Epithelium Carcinogenesis

Cross-talk between membrane and nuclear receptor signaling pathways has been suggested as an important mechanism governing respiratory epithelium carcinogenesis and sensitivity or resistance for all potential therapeutic interventions (5). In this vein, the identification of all crucial molecular “actors” in this functional model appears as a prerequisite for the development of novel pharmaceuticals or even for the optimal application of the already existing ones.

Nuclear receptors represent a large superfamily of ligand-dependent, DNA-binding, gene-specific transcription factors, albeit recent reports have also revealed ligand-independent actions (66). They are able to regulate decisive events during development, control cellular homeostasis, and inhibit or induce cellular proliferation, differentiation, and apoptosis. About 70 nuclear receptors have been identified to date, and with some notable exceptions, all members display an identical structural organization comprising a variable NH<sub>2</sub>-terminal domain, a well-conserved DNA-binding domain (crucial for recognition of specific DNA sequences), a linker region with central role in protein-protein interactions with transcription cofactors, and a COOH-terminal ligand-binding domain. Nuclear receptors bind as homodimers and/or heterodimers, along with the promiscuous heterodimerization partner retinoid X receptor, to stretches



and has been found to be repressed in normal respiratory epithelium (79). Based on the aforementioned “switch on/off” model, although one class of retinoid X receptors are usually overexpressed from the early stages of respiratory epithelium carcinogenesis (representing a possible protective cellular event), their inability to form heterodimers with other nuclear receptors, such as peroxisome proliferator-activated receptors (PPAR), might contribute indirectly to cyclooxygenase-2 overexpression through AP-1–dependent transcription, resulting in the inhibition of apoptosis (refs. 5, 67; see Fig. 3). Although the role of PPARs in the development of respiratory epithelium carcinomas has not been extensively investigated, several studies have shown that their activation can inhibit growth and enhance apoptosis of cancer cells (80, 81).

The tumor suppressor protein phosphatase and tensin homologue deleted on chromosome 10 (PTEN) modulates apoptosis by activating Akt (82). Recent data revealed that *PTEN* expression was associated with longer survival, whereas loss of *PTEN* was an independent poor prognostic factor for patients with respiratory epithelium carcinomas (83). Functional loss of *PTEN* results not only from physical loss of the *PTEN* gene but also from other mechanisms, particularly promoter aberrant methylation (84), whereas it seems that AP-1 proteins are involved in apoptosis through regulation of PTEN function (ref. 85; see Fig. 3). Furthermore, recent findings also suggested that PTEN may inhibit the insulin-like growth factor-1 (IGF-1) network, in either Akt-dependent manner (83) or through cross-talk with nuclear receptors (86). The functions of IGF-1 as a mitogen and antiapoptotic factor are well documented (87). Estrogen status is a recognized risk factor for lung cancer in women, as it is in the development of adenocarcinoma of the breast, endometrium, and ovary (87). Taioli and Wynder (88) first presented evidence that exogenous and endogenous estrogens may play a role in the development of lung cancer, particularly adenocarcinoma, among women. The cellular response to estrogen is mediated by estrogen receptor  $\alpha$  (ER $\alpha$ ) and ER $\beta$ , which are encoded by distinct genes and display a differential tissue distribution. Normal breast tissue exhibits expression of both ER $\alpha$  and ER $\beta$ , whereas in respiratory epithelium ER $\beta$  seems to be the dominant form (66). ER is known to mediate gene transcription via AP-1 enhancer elements as well as the well-established estrogen response elements. Investigations of AP-1–mediated trans-activation through ER have been done with rather complex promoters, such as *IGF-1*. Preclinical results imply that ER $\alpha$  and ER $\beta$  may function in opposition, with ER $\beta$  actually suppressing the function of ER $\alpha$  in AP-1–mediated trans-activation (89). ER $\beta$  has been shown to be expressed in both normal lungs and in lung tumors. It has also been reported that ER $\beta$  displays higher expression in lung adenocarcinomas than in squamous cell carcinomas (90, 91). ER $\beta$  expression in the lung has also been shown to correlate with the expression of certain carcinogen-metabolizing enzymes (92). In addition, further to the classic estrogenic effects in the nucleus, it is increasingly clear that ER signaling effects may take place in a ligand-independent manner, via cross-talk with growth factor receptors (e.g., EGFR and IGF-1R) in the plasma membrane (93). Estrogen and IGF-1 are potent mitogens that are involved in a wide array of processes, which control proliferation and differentiation in mammalian

cells. Both act through receptor-mediated signaling pathways. The cross-talk between these two signaling pathways is currently under intense investigation in respiratory epithelium carcinogenesis, whereas the role of AP-1 proteins in this interaction seems to be of paramount importance (see Fig. 3).

Remarkable progress in the area of gene control mechanisms has begun to unravel the transcriptional circuitries that operate in respiratory epithelium carcinogenesis. Different classes of gene-specific transcription factors, along with important cofactor complexes, comprise an intricate multi-protein interplay, which results in specific events in the nucleus producing different molecular abnormalities in various stages of carcinogenesis. Therefore, future research efforts should be focused on the identification of the key “players” that modulate the final steps of these complex molecular interactions. AP-1 seems to represent such a promising target.

### AP-1 as a Potential Treatment Target in Respiratory Epithelium Carcinogenesis

Genetic animal models and *in vitro* studies have shown that aberrant activation of AP-1 proteins is causally linked to pathogenesis, indicating that an abnormal expression and/or activation of AP-1 constituents by toxins can lead to disease development (94–99). Several investigations have documented that environmental toxicants like tobacco smoke, asbestos, silica, or other particulates lead to the development of respiratory diseases, including cancer, and that this is accompanied by an increase in AP-1 proteins expression in the exposed airway epithelia (94, 100). In cultivated bronchial epithelial cells exposed to cigarette smoke, AP-1 induction was also found to require a functional EGFR-MAPK pathway (101).

In clinical respiratory epithelium carcinomas, the results concerning the role of AP-1 are controversial. Jun and Fos expression seems to be variable between different normal and malignant cell lines. Consistent with *in vivo* observations, RNase protection analysis revealed high-level expression of c-Jun, JunB, JunD, and Fra-2 in the nontransformed mouse alveolar epithelial cell line C10, whereas the expression of c-Fos, FosB, and Fra-1 was very low or undetectable (102). However, detectable amounts of c-Fos and Fra-1, in addition to c-Jun, JunB, and JunD, were noticed in normal human bronchial epithelial cells (100). Intriguingly, malignant respiratory epithelial cells variably express AP-1 components. One study showed significantly lower levels of JunB, c-Fos, and Fra-1 mRNA in malignant cells compared with normal cells (103). In contrast, a different study showed a high but variable expression of c-Jun, JunB, JunD, and c-Fos mRNA in various human lung cancer cell lines (104). Immunohistochemical analysis of various neoplastic human lung tissues revealed a high level of expression of c-Jun antigen in atypical, hyperplastic, and metaplastic epithelium, whereas its expression in surrounding normal bronchial and alveolar epithelia was marginal or undetectable (105). Regarding c-Fos, in immunohistochemical studies, squamous cell lung carcinomas with c-Fos protein overexpression were shown to be more tumorigenic in nude mice, and the corresponding patients had a significantly shorter survival in multivariate analysis (106). In an immunohistochemical analysis of 21 possible prognostic indicators, c-Fos turned out as the strongest predictor of short survival in NSCLC (107). Interestingly, c-Fos overexpression is more

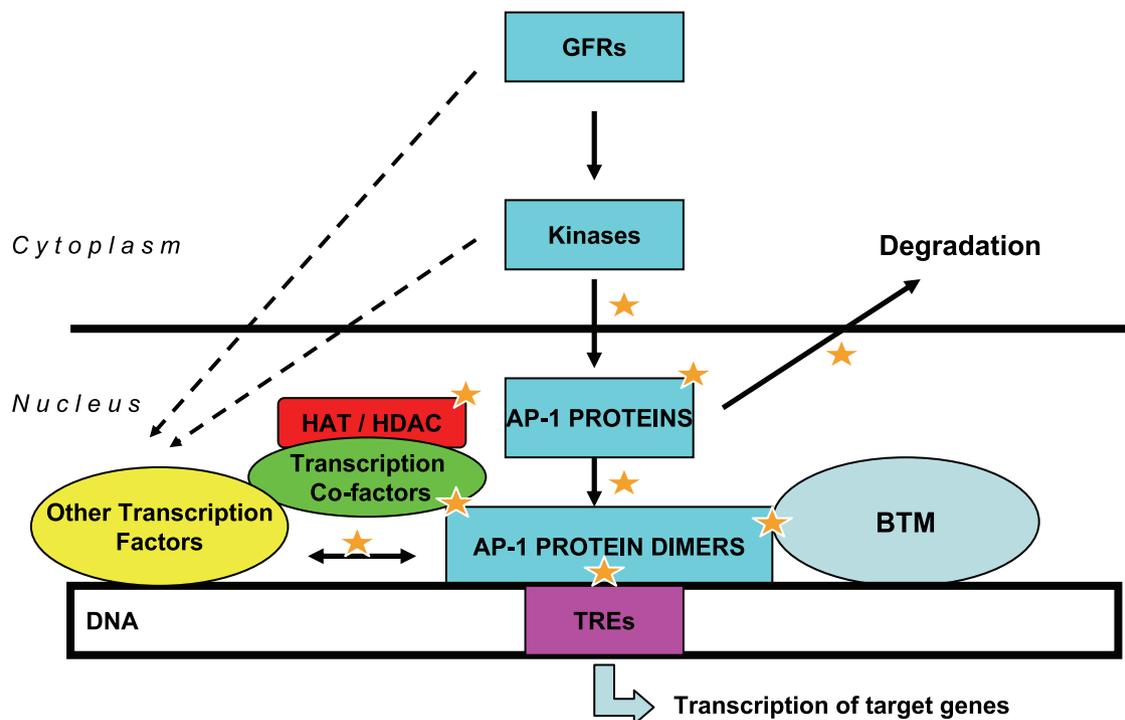
frequently found in tumors from smokers than in carcinomas from nonsmokers (108). Additional Fos family members were not analyzed in these studies. Yet, in an experimental system, the transformation of SCLC cells to a NSCLC phenotype was accompanied by expression of Fra-1 (109).

Early studies suggested that Fra-1 and Fra-2, due to their lack of a trans-activation domain, which is characteristic of c-Fos and FosB, might exert inhibitory actions on tumor cell growth (38). Nevertheless, recent data point to a positive effect of Fra-1, and partly Fra-2, on tumor invasion and progression in many tumor types (110, 111). Moreover, a model was proposed in which both Fra-1 and c-Fos act as adaptors for other transcription factors, or as transcriptional repressors rather than transcriptional activators (112). Alternatively, it is possible that a protracted induction of Fra-1 by mitogens and/or toxicants alters the dynamics of AP-1 by changing dimer composition (40). This might influence, either positively or negatively, the transcriptional activation of target genes, thereby playing a regulatory role in gene expression involved in respiratory defense mechanisms (113). Fra-1 might also be a valuable target for therapy. Some tumor-preventing agents function by deregulating Fra-1 expression in model systems (e.g., curcumin; ref. 114). Yet, most data concerning the function of Fra-1 in respiratory epithelium carcinogenesis are based on experimental results, and the role of these transcription factors in clinical tumors is still obscure (110). In most of the tumor tissues analyzed thus far, Fra-1 expression has been found far below the protein amounts detected in undifferentiated cell lines, and the electrophoretic mobility of the Fra-1 protein indicates that it is not highly phosphorylated, which might lead

to its stabilization and *in vitro* activation (40). Whether the low Fra-1 amounts in tumors have a similar effect to that seen in experimental systems, or if Fra-1 expression in single cells or cell clones within the tumors contributes to local invasion and metastasis, should be further explored.

The modular architecture of AP-1 proteins makes them vulnerable to the action of various treatment strategies (see Fig. 4). A candidate AP-1-directed drug may exert its action by interacting specifically with the DNA-binding/dimerization domain, the trans-effecting domain, or another domain/region that regulates a defined biochemical function (5). Anthocyanins (peonidin 3-glucoside and cyanidin 3-glucoside) have been shown to exert inhibitory effect on the DNA-binding activity and the nuclear translocation of AP-1 proteins (115). Alternatively, a candidate remedy might affect crucial conformational changes and interfere with the formation of the functional dimer species of AP-1 proteins. For instance, curcumin and its synthetic derivatives have been found to be able to suppress the formation of DNA-Jun-Fos complexes (116), whereas synthetic peptidic compounds are also pursued (e.g., SP600125; ref. 117).

Another potential way of modulating AP-1 proteins is through hampering the activity of upstream effector molecules that regulate their function. The majority of these molecules are protein kinases, the most relevant being elements of the MAPK pathway (118). A wide gamut of natural and/or synthetic agents interfering with this pathway are under evaluation, such as ascochlorin and silibinin targeting extracellular signal-regulated kinase 1/2 (119, 120); flavonoids (kaempferol and genistein) that hinder JNK and extracellular signal-regulated kinase,



**FIGURE 4.** Small-molecule drugs targeting AP-1 network could influence its action at multiple levels (orange asterisks). However, the rational design of these compounds and their optimal use as preventive or treatment regimens postulate the in-depth understanding and identification of AP-1 molecular features and interactions with other signaling molecules in the various stages of respiratory epithelium carcinogenesis.

respectively (121); resveratrol aiming at MAPK kinase 1 (122); and various other antioxidants (123, 124). Tackling of these kinases with selective inhibitors might also represent an effective approach in lung cancer therapeutics (125). The Ras-MAPK pathway represents one site of regulatory convergence; therefore, its constituents appear as suitable candidate targets for indirect AP-1 therapeutic manipulation. Three components of this pathway have received, thus far, most of the scientific interest as potential targets for pharmacologic intervention: Ras, Raf, and MAPK kinase. Several novel agents targeting these proteins are in preclinical and early clinical evaluation in a variety of human tumors, including lung cancer (126-128). Among all AP-1-related kinases, phosphorylation by JNKs is currently considered the most important positive regulator of c-Jun stability (129). However, although JNK-specific inhibitors (117) and short interfering RNAs directed against JNK1/2 (78) are being designed and appear as attractive anti-AP-1 agents, many aspects of their molecular action during respiratory epithelium carcinogenesis have to be unveiled, such as stage-specific action of various JNK isoforms, before they could be considered as valuable treatment strategy (130).

JNK-mediated c-Jun phosphorylation prevents the ubiquitin-dependent degradation of c-Jun, and this phosphorylation-triggered stabilization contributes to the efficient activation of c-Jun following exposure to a plethora of external stimuli. Currently, JNK2 is the only known kinase that functions as a negative regulator of the ubiquitin-dependent degradation of c-Jun under normal growth conditions (131). On the other hand, the positive regulation of the ubiquitin-dependent degradation of c-Jun might be beneficial in lung carcinogenesis because, through maintaining low steady-state levels of c-Jun, inhibition of c-Jun-driven cell transformation might be feasible. In accordance to this assumption, it was recently documented that COOH-terminal Src kinase binds to and phosphorylates c-Jun, and that this phosphorylation promotes c-Jun degradation, thereby inhibiting AP-1 activity (132). Combined with the fact that low levels of COOH-terminal Src kinase have been detected in various malignant tumors, this protein might represent an attractive indirect way of AP-1 therapeutics (133).

AP-1 activity is also controlled by redox-dependent mechanisms (134). The reduced state of critical cysteine residues present in the DNA-binding domain of AP-1 proteins has been found to be essential for DNA binding (135). Some naturally occurring chemopreventive and/or chemotherapeutic agents, such as sulforaphane, bioactive components of garlic, zerumbone, curcumin, "antagonist G," and others, exert their effects through oxidation or covalent modification of thiol groups (136, 137). Therefore, they could be used as AP-1-directed agents to suppress the aberrant overactivation of carcinogenic signal transduction, or restore/normalize or even potentiate cellular defense signaling routes.

The AP-1 cofactor network represents another potential level of targeting, with the aim being a nonfunctional interaction of AP-1 proteins with their partners in a given transcriptional complex. Thus, gene expression could either be decreased (e.g., inactivation of a transcription coactivator) or increased (e.g., activation of a transcription corepressor). This type of targeting might be either direct or indirect. For example, Jun

activation domain-binding protein 1 (Jab1) is an AP-1 coactivator that interacts and potentiates trans-activation by c-Jun, hence promoting cellular proliferation and apoptosis modulation during lung carcinogenesis (138). Recently, it was shown that Jab1 overexpression correlates with poor outcome in patients with lung cancer, and that this protein might represent a rational therapeutic target (139).

The implication of epigenetics in carcinogenesis is now considered determinative. As is the case in most solid tumors, respiratory epithelium carcinogenesis is governed by the repression of tumor suppressor genes and/or activation of oncogenes. DNA methylation is tightly linked to respiratory epithelium carcinogenesis (28). Various genes have been shown to be hypermethylated in lung cancer, among them *p16* and *RASSF1A* (140). It has been shown that the products of these genes are capable of inhibiting JNK (141, 142). To this end, an intriguing hypothesis might be that the application of demethylating agents could restore the activity of these proteins, thus achieving JNK inhibition. Histone posttranslational modifications and especially acetylation/deacetylation are also recognized as important regulators of the transcriptional control of many genes. Three major families of histone deacetylases have been identified, and multiple mechanisms seem to engage them in cancer development. It has been reported that the corepressors N-CoR and SMRT cooperate with histone deacetylases and inhibit JNK pathway, thus providing an alternate strategy of AP-1 indirect therapeutics (143).

Cross-talk interactions between AP-1 proteins and other signal transduction pathways are gradually being elucidated (see Fig. 3) and might constitute the basis for new treatment rationales. For example, it has been documented that down-regulation of RAR $\beta$  expression accompanied by AP-1-enhanced expression and activity represent early events during respiratory epithelium carcinogenesis, contributing both to the suboptimal results of the currently used retinoids and to unopposed AP-1-driven cellular proliferation (5). The cause of RAR $\beta$  down-regulation has been attributed to both genetic (144) and epigenetic mechanisms (145). Thus, it seems reasonable to apply various strategies to restore the well-recognized AP-1 trans-repressing property of RAR $\beta$  (146). Such approaches might be epigenetic targeting of RAR $\beta$  (147), modulation of critical participants in this interaction (e.g., CBP/p300; ref. 148), and combination with the aforementioned AP-1-directed therapeutics (e.g., JNK inhibition).

The role of AP-1 in apoptosis-related interacting pathways is also progressively being unraveled. Nuclear factor- $\kappa$ B (NF- $\kappa$ B)/PPAR $\gamma$  and/or AP-1/PPAR $\gamma$  functional "on/off" switches are considered crucial molecular events during lung carcinogenesis (67, 149, 150). NF- $\kappa$ B/Rel transcription factors have emerged as important regulators of cell survival (151). Activation of NF- $\kappa$ B antagonizes programmed cell death induced by tumor necrosis factor and several other stimuli (151), whereas this inhibiting activity is thought to be mediated through sustained activation of the JNK cascade (152). A proof of this interaction was the recent identification of TAM67 (a dominant-negative c-Jun mutant) that impairs both AP-1 and NF- $\kappa$ B (153). The ability of PPAR $\gamma$  to modulate gene expression requires its heterodimerization partner retinoid X receptor (154). Regulation of PPAR $\gamma$  activity along with transcription cofactors

(e.g., CBP/p300) can influence NF- $\kappa$ B and AP-1 transcriptional potentials, leading to up-regulation of cyclooxygenase-2 and apoptosis inhibition (67, 155). Therefore, the combined application of selective PPAR $\gamma$  ligands, cyclooxygenase-2 inhibition, and NF- $\kappa$ B therapeutics might offer efficient blockade of AP-1-associated activity. In this regard, it has been shown that nonsteroidal anti-inflammatory agents (e.g., sulindac) might have multiple and synergistic negative effects on AP-1 signaling, as they can inhibit both JNKs and cyclooxygenase-2 actions (156). Moreover, recent data indicate that other members of PPAR family (e.g., PPAR $\alpha$ ) could also interfere with AP-1 activity (157), providing additional choices of pharmaceutical targeting. Various PPAR $\alpha$  (e.g., GW 9578) and PPAR $\gamma$  modulators (e.g., troglitazone, rosiglitazone, pioglitazone, ciglitazone, GW 1929, GI 262570, GW 0207, and GW 7845) as well as dual receptor agonists (e.g., KRP-297 and JTT-501) have shown promising antiproliferative and chemoprevention activity *in vitro* and *in vivo* (158). The cross-talk between IGF-1R and ER $\beta$  signaling pathways has been also suggested to hold important role regarding AP-1-driven growth stimulation and apoptosis relieve during lung carcinogenesis, mainly by affecting downstream molecules and their associated pathways (87). Thus, manipulation of this interaction by ER $\beta$ -selective agents might constitute a potentially effective indirect AP-1 therapeutic strategy.

### Concluding Remarks: Outlook

AP-1 proteins consist a class of sequence-specific transcription factors with pivotal role in respiratory epithelium development and carcinogenesis. The detailed molecular features of these proteins are gradually elucidated. The most important characteristics of their mode of action are their intense interplay with other crucial signal transduction pathways that participate in respiratory epithelium carcinogenesis as well as the great diversity regarding the formation of different dimers in normal, premalignant, and malignant respiratory epithelial lesions upon the influence of various mitogenic stimuli.

In-depth mapping of the immensely complex signaling networks culminating in AP-1 proteins will provide new insights about their precise role in gene transcription during respiratory epithelium carcinogenesis. Innovative AP-1 structure/function-based small-molecule drugs will be created in the near future, with increased selectivity and minimal side effects, by pinpointing the nuclear partners that “orchestrate” AP-1 contribution to respiratory epithelium carcinogenesis. Functional genomics and proteomics hold key positions in this scenario, whereas pharmacokinetics problems that represent a major limiting step in drug development have to be adequately addressed to overcome the difficulties of nuclear-directed therapeutics (e.g., nanotechnology application in cancer therapeutics).

As transcription alone does not fully correlate with all genetic/epigenetic abnormalities of respiratory epithelium cancers, biologically targeted anticancer agents should tackle several different cellular processes. The optimal sequence of such a combinatorial, molecularly “tailored” treatment of carcinogenesis represents the most promising, albeit not yet possibly applied, approach of chemoprevention and/or treatment of respiratory epithelium neoplasms.

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