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European Journal of Cancer 41 (2005) 2449–2461

European Journal of Cancer

www.ejconline.com

The Fos family of transcription factors and their role in tumourigenesis

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Available online 30 September 2005

Abstract

Members of the Fos family (c-Fos, FosB and its smaller splice variants, Fra-1 and Fra-2) dimerise with Jun proteins to form the AP-1 transcription factor complex. Based on the rapidly growing amount of data from experimental studies, animal models and investigations on clinical tumour samples, this review summarises the current knowledge about the role of these proteins in carcinogenesis. In addition to c-Fos, which has oncogenic activity and is frequently overexpressed in tumour cells, Fra-1 seems to play a role in the progression of many carcinomas. The results obtained from various studies show different implications for these transcription factors according to tumour type, i.e., Fra-1 overexpression enhances the motility and invasion of breast and colorectal cancer cells, but inhibits the tumourigenicity of cervical carcinoma cell lines. Knowledge about regulation of invasion and metastasis in different malignant tumours *in vivo* might open promising perspectives to targeted therapeutic approaches.

Keywords: c-Fos; FosB; Fra-1; Fra-2; Cancer; AP-1; Invasion; Transformation

1. Introduction

The Fos family of transcription factors includes c-Fos (the human homolog of the retroviral oncogene v-Fos), FosB, Fra-1 and Fra-2 as well as smaller FosB splice variants FosB2 and deltaFosB2. Together with Jun family members (c-Jun, JunB and JunD) they form the group of AP-1 proteins which, after dimerisation, bind to so-called TPA-responsive elements (TRE's; TGAC/GTCA) in the promoter and enhancer regions of target genes. Since TRE-containing promoter constructs are strongly activated by the tumour promoter TPA [1] and the first AP-1 proteins (c-jun and c-Fos) to be discovered were found to be transforming in NIH3T3 rat fibroblasts [2], the AP-1 complex was implicated in carcinogenesis soon after discovery. In contrast to Jun proteins, Fos family members are not able to form homodimers, but hetero-dimerise with Jun partners, giving rise to various trans-activating or trans-repressing complexes with different biochemical properties. *In vitro* studies have shown that Jun–Fos heterodimers are more stable and have stronger DNA-binding activity than Jun–Jun homodimers [3,4]. In F9 teratocarcinoma cells, c-Fos enhanced the trans-activating and transforming properties of c-Jun and JunB [5]. Thus, the expression of Fos proteins might be crucial for the activity of AP-1-regulated genes.

All AP-1 proteins are characterised by a basic leucine-zipper region for dimerisation and DNA-binding. Yet, while c-Fos and FosB proteins harbor a C-terminal transactivation domain, Fra-1, Fra-2 and FosB2 lack this region (Fig. 1). Accordingly, these proteins are not transforming in rat fibroblasts, and an inhibitory function of these factors on AP-1 activity has been proposed [6]. Yet, recent results which will be described in this review suggest that in many tumours, these non-transforming Fos proteins, especially Fra-1 and Fra-2,

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doi:10.1016/j.ejca.2005.08.008

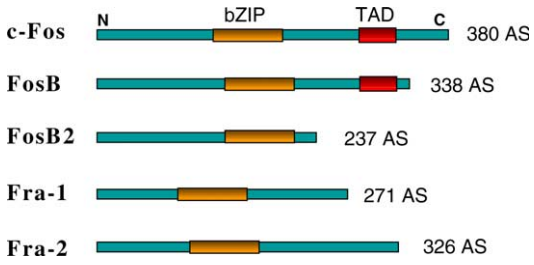

Fig. 1. Schematical presentation of the structure of Fos proteins. AS, amino acids; bZIP, basic leucine zipper region for dimerisation and DNA-binding; TAD, C-terminal transactivating domain [7].

might be involved in the progression of many tumour types. Upon stimulation of fibroblasts by serum, c-Fos and FosB are rapidly and transiently induced, whereas Fra-1 and Fra-2 expression is delayed and more stable. This is probably achieved by activation of the Fra-1 and Fra-2 promoters by Jun/Fos dimers [7]. The activity of all Fos family members is also modulated by post-translational modification: phosphorylation by different kinases, i.e., MAPK, cdc2, PKA or PKC influences protein stability, DNA-binding activity and the trans-activating potential of the transcription factors [8–10].

AP-1-regulated genes include important regulators of invasion and metastasis; proliferation, differentiation and survival; genes associated with hypoxia; and angiogenesis (Fig. 2). For a more detailed description of the current knowledge in this field based on experimental data, the reader is referred to some excellent review articles [7,11–14]. Many oncogenic signalling pathways converge at the AP-1 transcription factor complex. The specific influence of a specific AP-1 protein on a promoter depends on the dimer partners, the promoter architecture as well as other transcription factors and co-activators acting on the promoter [11]. Therefore, one AP-1-regulated gene might be preferentially induced

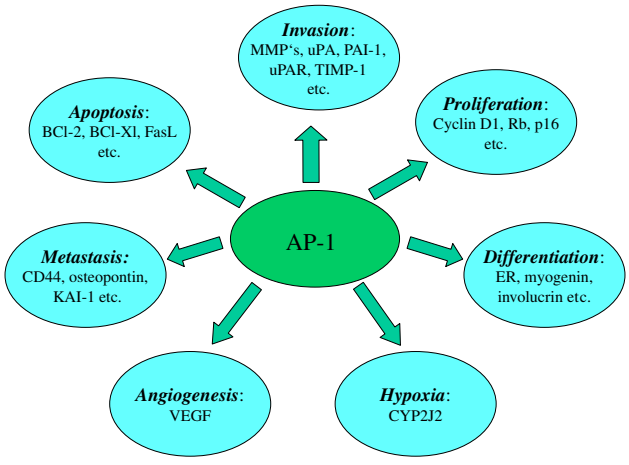

Fig. 2. AP-1-responsive genes in cancer.

by Jun–c-Fos, while another gene is mainly induced by JunD/Fra-1 dimers. 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 or Jun–ATF dimers [11]. Generally, Fos family members have both overlapping as well as unique roles, and function in a tissue-specific way. Regarding these results, the measurement of AP-1 activity using artificial AP-1-regulated promoter constructs which was performed in many early studies on cancer cells is not very informative, since this activity does not reflect the biological behavior of cancer cells. Therefore, more recent studies have included the analysis of expression and/or activity of all Jun and Fos family members. Using this approach, it was demonstrated in several experimental systems that malignant transformation and progression is accompanied by a cell-type specific shift in AP-1 dimer composition (see below). The large amount of experimental data on this issue is in sharp contrast to only few reports dealing with the expression or activity of AP-1 proteins in human clinical tumour tissues. In the following sections, the experimental and *in vivo* data regarding the function of Fos family members in carcinogenesis are summarised.

2. Role of Fos proteins in tumour cells and clinical tumour tissues

2.1. Bone and chondroid tumours

The Finkel–Biskis–Jinkins murine osteogenic sarcoma virus, a retrovirus encoding the viral *v-Fos* oncogene, induces osteosarcomas when injected in rodents, indicating that bone tissues might be the primary target of this oncogene. This was confirmed by the observation that overexpression of c-Fos from a class I MHC promoter in transgenic mice results in the development of osteosarcomas due to increased proliferation of osteoblasts whereas ectopic expression of the other Jun and Fos proteins did not induce any malignant tumours in rodents [15]. Activation of the *c-Fos* transgene in mice results in a strong cyclin D1 overexpression in osteoblasts and chondrocytes, which might contribute to uncontrolled proliferation of these cells [16]. Yet, in mouse osteoblast cell lines with inducible c-Fos expression, the accelerated growth after induction was accompanied by increased cyclin E and cyclin A expression, leading to enhanced activity of the cyclin-dependent kinase cdk2 and rapid S-phase entry, and deregulated cyclin A expression was also found in Fos-induced mouse osteosarcoma cells *in vivo* [17].

Due to these results, human osteosarcomas were analysed for c-Fos expression in various studies. Positive, but mostly weak staining with immunohistochemistry (IHC) protocols was found in 40–50% benign osteomas

and osteoblastomas, whereas in conventional osteosarcomas, c-Fos expression was more frequent (67% of the cases) and generally more intense [18]. In addition, associations of c-Fos overexpression with high-grade lesions [18,19] and with a higher frequency of relapse were described [20]. Moreover, Kakar and colleagues [21] reported a predictive value of c-Fos expression for outcome after chemotherapy, since 8/8 cases with poor response, but only 1/5 cases with good response were c-Fos-positive with IHC.

The role of c-Fos in chondroid neoplasia is still unresolved. Chimeric mice generated using embryonic stem cells with high exogenous c-Fos expression developed cartilage tumours at 3–4 weeks of age, and a strong c-Fos overexpression was detected in these tumours [22]. This suggested that the oncogene might also be involved in chondroid carcinogenesis. Yet, investigations on human neoplasias gave controversial results: in an IHC study, human chondrogenic lesions were always c-Fos-negative in contrast to osteogenic tumours [18]. In contrast, *in situ* hybridisation showed moderate or high c-Fos mRNA expression not only in 50% osteosarcomas, but also in 4/6 chondrosarcomas and 90% (23/26) fibrous bone lesions [23].

2.2. Breast cancer

Mammary carcinomas are probably the most intensively studied tumours with respect to the role of AP-1 family members in cancer.

Interesting experimental results were obtained from studies using two mouse mammary adenocarcinoma cell lines derived from the same tumour: the weakly invasive and non-metastatic CSML0 cells and the highly metastatic CSML100 cells. Regarding the Fos proteins, both cell lines differed significantly: in CSML0 cells, only c-Fos expression was detected, whereas in the metastatic cells, strong Fra-1 and Fra-2 expression was found, and c-Fos and FosB were undetectable. There was no difference in these cell lines with respect to the expression of Jun proteins. In an analysis of mouse adenocarcinoma cell lines, Fra-1 positivity correlated with a highly malignant, E-cadherin-negative and mts1-positive phenotype. Opposite results were obtained for c-Fos, whereas Fra-2 expression did not show any correlations with the malignant behavior of tumour cells. In stably transfected CSML0 cells showing conditional Fra-1 expression, induction of Fra-1 resulted in an elongated cell shape, a higher invasive potential and enhanced motility [24].

In additional transfection experiments with retroviral vectors coding for c-Fos, Fra-1 and Fra-2, specific effects on the expression of AP-1 target genes involved in invasion and metastasis were found in CSML0 cells: c-Fos and Fra-1, but not Fra-2 overexpression resulted in the activation of uPA, PAI-1, uPAR, mts1 and HMGI(Y). In contrast, all three transcription factors (c-Fos, Fra-1, Fra-2) induced the expression of osteopontin (OPN), thrombospondin and CD44 which are involved in metastasis in human mammary tumours [25]. MMP9 expression was not activated by these factors despite an AP-1 element in its promoter. Both Fra-1 and c-Fos overexpression resulted in morphological changes leading to an elongated shape and enhanced motility. For Fra-2, a cell-type specific function was detected with no effect on motility in CSML0 cells, but with a positive effect on motility of a fibroblastoid cell line [26].

Similar effects on motility and invasion were observed in human breast cancer cell lines: in a cDNA array study with four highly invasive and nine weakly invasive human breast cancer cell lines, 24 genes were identified which were differentially expressed: among them, c-Jun and Fra-1 expression levels were significantly enhanced in the highly invasive cells [27]. Therefore, the authors suggest that Fra-1 might be a valuable diagnostic marker in breast cancer.

The most extensively studied human breast cancer cell lines are the poorly invasive, ER-positive, epitheloid MCF7 cells, and the ER-negative, highly invasive, fibroblastoid-shaped MDA-MB231 cells. They differ in the expression of FosB, which is only expressed in MCF7 cells, and Fra-1, which is only detectable in MDA-MB231 cells [28,29]. In transient transfection experiments, the motility of MCF7 cells was significantly enhanced by c-Fos and FosB, whereas invasion through matrigel was increased by Fra-1 and, less strongly, c-Fos and FosB. In contrast, the motility of MDA-MB231 cells was not further increased by overexpression of Fos proteins, but invasion of these cells was increased by Fra-1 and, less efficiently, Fra-2 [30]. In a recent study on the same cell lines, Fra-1 expression was increased in MCF7 cells by stable transfection, and down-regulated in MDA-MB231 cells by siRNA [31]. Overexpression of Fra-1 in MCF7 cells had no influence on proliferation in medium containing 10% serum, but enhanced the growth rate in medium with 1% serum. In addition, cell motility and invasion were increased by about 2-fold. The effects of Fra-1 silencing in MDA-MB231 cells were even stronger with drastically reduced proliferation in 10% and 1% serum, and 2–3-fold reduced motility and invasion. By microarray experiments and subsequent Western blot analysis, five genes were identified whose expression was strongly (MMP-9, MMP-1, VEGF) or weakly (TIMP1, Cyclin D1) upregulated in cells showing Fra-1 overexpression. Obviously, Fra-1 influences not only cell proliferation (by cyclin D1) and invasion (MMP9, MMP1, TIMP1), but, by regulating the potent angiogenic factor VEGF, also angiogenesis as a prerequisite for efficient tumour growth and invasiveness. The effect of Fra-1 on MMP1 and MMP9 expression was a direct effect since the activity of the respective promoters in reporter

constructs was increased by Fra-1. This is in contrast to the results obtained by us [30] and others [24] who found no MMP9 promoter activation by Fra-1 and Fra-2 in mammary carcinoma cells. Similar to observations in colon cancer cells [32], Fra-1 obviously influences motility and invasiveness by downregulating β1-integrin leading to inactivation of the Rho-ROCK pathway and decreased cell-substratum adhesion [31].

In studies on clinical breast cancer tissue samples, we found strong variations in AP-1 protein expression. In Western blots, the most interesting results were observed for FosB, Fra-1 and Fra-2. FosB expression in breast cancer samples was significantly associated with a positive estrogen receptor status and a well-differentiated tumour type (grade 1–2) [28]. By immunohistochemistry, these results could be confirmed, and FosB positivity also correlated with strong progesterone and weak/absent HER2/neu staining [33,34]. FosB protein and mRNA expression was detected in normal epithelial cells of mammary lobules and ducts, and in well-differentiated tumours, whereas it was undetectable or weak in most poorly differentiated, steroid-hormone receptor-negative carcinomas. The results of sequencing and RT-PCR indicated that FosB is not inactivated by mutation in these cases, and that the downregulation of FosB in undifferentiated cells takes place mainly on a post-transcriptional level [34]. Although both the full-length FosB mRNA and the shorter mRNA splice variant coding for FosB2 were detected by RT-PCR, only FosB protein was detectable in Western blots, indicating different stabilities of the protein products.

A strong inverse correlation was observed for the expression of FosB and Fra-1 in breast cancer cell lines and tumour tissues [28]: in most FosB-positive tumours, Fra-1 was undetectable in Western blots, whereas it was positive in FosB-negative cases. Yet, compared with the strong Fra-1 expression in the ER-negative cell lines MDA-MB231 and HBL-100, only weak Fra-1 bands were observed in undifferentiated tumours. In addition, the position of the bands points to a stronger phosphorylation of the Fra-1 protein in cell lines.

Since many cell-cycle regulating genes are partly controlled by AP-1, the same breast cancer samples were also analysed for expression of six cell-cycle regulating genes as well as the proliferation marker Ki67. Significant correlations of high Ki67 positivity with weak/negative FosB and positive Fra-1 immunoreactivity point to an involvement of these transcription factors in cell-cycle regulation. Regarding the cell-cycle promoting proteins, we found correlations of high c-Fos, Fra-1 and Fra-2 expression with high cyclin E protein levels, and of cyclin D1 and Fra-2 expression [35]. In contrast, FosB positivity was associated with higher expression of the cell-cycle inhibitory retinoblastoma protein Rb. Obviously, the effect of FosB on cell proliferation is opposite to that of c-Fos, Fra-1 and Fra-2. Cyclin D1 and Rb are AP-1 target genes, whereas cyclin E transcriptional activation is mainly activated by E2F binding sites in the promoter. Therefore, the reason for the correlations between cyclin E and Fos proteins is still unknown.

In the same way, correlations were also performed with the expression of the AP-1 regulated proteases MMP1 and MMP9 and the invasion-associated plasminogen activator inhibitor PAI-1. Similar to the results obtained with the cell-cycle regulators, the results point to opposite functions of FosB and the other Fos family members: significant correlations were found for MMP1 with FosB, whereas MMP9 expression was associated with c-Fos, Fra2, and the presence of the more slowly migrating, phosphorylated Fra-1 band [30]. This association is not achieved by direct activation of the MMP9 promoter, since promoter activity is not increased in the two cell lines by Fra-1 and Fra-2 in cotransfection experiments. For PAI-1, only correlations with Fra-2 were found. A comparison with clinical data of patients resulted in statistically significant correlations of nodal involvement with high MMP9 and weak MMP1 expression and the presence of the phosphorylated Fra-1 protein. In addition, recurrence was more frequently observed in patients with strong Fra-2 expression. These data indicate that the development of an undifferentiated, strongly proliferating and invasive mammary tumour is accompanied by a shift in the expression of Fos family members not only in experimental systems, but also *in vivo*. Progression of mammary carcinomas obviously involves downregulation of FosB as well as upregulation and phosphorylation of Fra-1 and Fra-2.

A highly malignant subgroup of mammary carcinomas is inflammatory breast carcinomas (IBC). In a real-time RT-PCR study focusing on these tumours, Bieche [36] searched for genes which are upregulated in comparison with non-IBC carcinomas. Out of 538 genes which were included in the study, 27 were upregulated at the mRNA level in IBC, among them c-Fos and FosB. Yet, in a comparison of IBC patients who relapsed and those that did not relapse, AP-1 proteins were not discriminatory.

2.3. Endometrial carcinoma

Endometrial carcinoma frequently develops under conditions of unopposed estrogenic stimulation on the basis of endometrial hyperplasias. In normal endometrial cells, addition of estrogen results, via protein kinase C, in enhanced expression of c-Jun and c-Fos. In addition to the mostly well-differentiated endometrioid endometrial tumours, a minority of poorly differentiated, serous-papillary and clear cell carcinomas which have often lost steroid hormone receptors arise independent of steroid hormones. According to Fujimoto [37], the c-Fos expression in these high-risk adenocarcinomas

is lower than in well differentiated tumours. Yet, additional studies indicate that c-Fos is no prognostic indicator in endometrial cancer: this was shown in an immunohistochemical study on 63 carcinoma samples [38] where c-Fos expression was not associated with clinical outcome, and by analysis of c-Fos mRNA expression, which did not correlate with tumour grade and stage [39] as well as steroid hormone receptor status and Ki67 expression [40].

In the first systematic analysis of all Jun and Fos family members in endometrial carcinoma, our group could show significant correlations of c-Fos overexpression in Western blots with high histological grading and a negative ER and PR status, which suggests a role of this AP-1 protein in tumour progression and de-differentiation [41]. Since AP-1 proteins are well-known regulators of cell proliferation, we compared their expression levels with expression of eight cell-cycle regulatory proteins which had been analysed before in the same tumour specimens. C-Fos expression correlated significantly with cell-cycle promoters (cyclins E and B1, cdk2 and cdk4) and cell-cycle inhibitors (p16, p21, Rb). The latter proteins are often paradoxically upregulated in human malignant carcinomas, indicating that their proliferation-inhibiting function can be overrun in tumour cells. In our study, the closest association was found between c-Fos and cyclin B1 which suggests a role of the transcription factor in the regulation of mitosis. In contrast to the results obtained with mammary carcinomas, FosB and Fra-1 were not associated with any prognostic parameters in endometrial cancer, but only with the tumour suppressors Rb (FosB) and p21 (Fra-1). Interestingly, Fra-2 overexpression was associated with high cyclin B1 levels and significantly more often found in non-endometrioid than in endometrioid tumours. This result points to different regulatory mechnisms in these histological subtypes and a replacement of c-Fos by Fra-2 in AP-1 complexes of non-endometrioid tumours [41].

2.4. Cervical cancer

In more than 90% of of the cervical carcinomas, the main etiological agent is infection with high-risk papillomavirus types (HPV 16, 18, etc.; [42,43]). Progression from weak cervical dysplasia (CIN1) to the invasive tumour is accompanied by an increased expression of the viral oncogenes E6/E7 which, by inactivating the cellular proteins p53 and Rb, interfere with the normal regulation of cell proliferation, differentiation, apoptosis and DNA repair [44]. Since the promoter driving E6/E7 expression harbors two (HPV18) or three (HPV16) AP-1 binding sites, the AP-1 proteins have an additional, important function in these tumours by regulating the viral oncogene expression.

In contrast to breast cancer, Fra-1 was shown to suppress tumourigenicity and malignant characteristics of cervical cancer cells. The antioxidative drug PDTC led to enhanced AP-1 binding, but suppressed viral gene expression in cervical cancer cells. This contradiction could be explained by the observation that incubation with the drug resulted in a re-organisation of the AP-1 complex by upregulation of c-Jun and Fra-1 and downregulation of c-Fos [45]. The tumour-suppressing function of Fra-1 was confirmed by the analysis of non-malignant hybrid cells derived from HeLa cells and normal fibroblast: in contrast to the malignant parental HeLa cells, the major Jun dimerisation partner in the hybrid cells was Fra-1, whereas conversion to tumourigenicity was accompanied by a re-expression of c-Fos and lower expression levels of Fra-1 [46]. Similar observations were made with somatic-cell hybrids derived from two tumourigenic cervical carcinoma cell lines (i.e., HeLa and SW756 or CaSki) which differed from their parental cells in tumourigenicity. Here again, non-tumourigenic clones showed strong Fra-1 and low c-Fos expression [47]. The loss of Fra-1 in tumourigenic cells is partly brought about by a decreased expression of the transmembrane receptor notch1 which is expressed in normal cervical keratinocytes, but strongly reduced in HPV-positive cancer cells. Re-transfected notch1 downregulates HPV-E6/E7 expression through upregulation of Fra-1 and suppression of AP-1 activity [48].

Recently published results obtained with cervical precancerous and cancer lesions point to the same direction [49]: in early precancer lesions (CIN1), a relatively low c-Fos and high Fra-1 expression with low overall AP-1 binding activity was observed, while c-Fos expression was high with absent Fra-1 immunoreactivity in invasive cervical cancer. Interestingly, the same authors could revert the expression levels of c-Fos and Fra-1 in the HeLa cell line by treatment with the natural antioxidant curcumin. This provides an explanation for its known anticarcinogenic properties and a basis for developing a new therapeutic approach.

2.5. Ovarian cancer

In ovarian carcinoma cell lines, c-Fos expression correlates with response to paclitaxel therapy in nude mouse xenografts [50] but confers cisplatin resistance in another experimental system [51].

Delivery of a dominant-negative Fos mutant, A-Fos, in cisplatin-resistant ovarian cancer cells led to a decrease in cell viability at cisplatin doses normally not lethal to the cells [52]. By CGH array analysis of 11 microdissected ovarian carcinoma samples, c-Fos amplification was observed in 6/11 cases, which was accompanied by overexpression in most tumours [53].

Other experimental data point a role of Fra-1 in ovarian carcinogenesis, i.e., the comparison of rat ovarian surface epithelial cells (ROSE) with daughter cells transformed by mutant K-ras oncogene. After subtractive suppression hybridisation, >200 differentially expressed genes were identified. Among them, Fra-1 was upregulated more than 100-fold in transformed cells [54]. Downregulation of Fra-1 by RNA interference in these cells reduced their proliferation rate by 50%. Other results indicate that Fra-1 might also be involved in motility and adhesion of ovarian cancer cells [55]. Overexpression of α(v)β3 integrin and subsequent attachment of OV-MZ-6 cells to vitronectin increased cell motility accompanied by changes in cell morphology. By cDNA microarray analysis, these cells showed altered Fra-1 expression.

2.6. Mesotheliomas

There is strong evidence that changes in AP-1 composition are involved in the development of asbestos-induced mesotheliomas. Exposure of rat pleural mesothelial cells to crocidolite asbestos fibers resulted in striking increases in Fra-1 protein levels both in cell extracts and in AP-1 complexes, whereas the expression of the other Fos family members was unaffected [56]. This induction of Fra-1 was dependent of activated extracellular-regulated kinases (ERK 1/2). Although Fra-1 overexpression alone was not sufficient for mesothelial cell transformation, it might be necessary for maintenance of the full transformed phenotype, because transfection of rat mesothelioma cells with a defective *fra-1* construct resulted in reversion to a normal morphology and a decrease in colony size and number in soft agar. In contrast, cell proliferation was not influenced by Fra-1. By cDNA microarray analysis of normal and asbestos-exposed rat mesothelial cells and mesothelioma cell lines, the same group confirmed the strong Fra-1 upregulation by asbestos fibers (9–10-fold) or in mesothelioma cells (>100-fold; [57]). These results were also validated in human mesothelial and mesothelioma cells. Interestingly, Fra-1 silencing by RNA interference resulted in decreased expression levels of CD44 and c-met, two cell surface receptors that are critical for growth and invasiveness of tumour cells.

2.7. Lung cancer

Several experimental studies have shown that environmental toxicants like tobacco smoke, asbestos, silica or particulates lead to the development of respiratory diseases including lung cancer, and that this is accompanied by an increase in c-Fos and/or Fra-1 expression in the exposed airway epithelia (reviewed by Reddy and Mossman, [58]). In cultivated bronchial epithelial cells exposed to cigarette smoke, Fra-1 induction needs a functional EGFR–MAPK pathway [59].

In clinical lung carcinomas, the results concerning the role of c-Fos are controversial. In a Northern blot analysis with RNA extracted from non-small cell lung carcinoma (NSCLC) specimens and adjacent normal tissue, c-Fos expression was significantly stronger in normal as compared to malignant tissues [60]. In contrast, in immunohistochemical studies, squamous cell lung carcinomas with c-Fos protein overexpression were shown to be more tumourigenic in nude mice, and the corresponding patients had a significantly shorter survival in multivariate analysis [61,62]. In an IHC analysis of 21 possible prognostic indicators, c-Fos turned out as the strongest predictor of short survival in NSCLC [63]. Interestingly, c-Fos overexpression is more frequently found in tumours from smokers than in carcinomas from non-smokers [64]. Additional Fos family members were not analysed in these studies. Yet, in an experimental system, the transformation of small cell lung carcinoma (SCLC) cells to a NSCLC phenotype is accompanied by expression of Fra-1 [65].

2.8. Colorectal cancer

Experimental data suggest an important role of Fra-1 in the regulation of the malignant potential of colorectal cancer cells. Fra-1 expression levels are upregulated by activated K-ras which leads to survival of the normally adherent cells in suspension [32,66]. Signalling from ras to Fra-1 is through raf, MEK, and the extracellular-signal-regulated kinases ERK1/2. These MAP kinases are able to phosphorylate Fra-1 [8], leading to its protection from proteosomal degradation. By siRNA experiments, Vial and co-workers [32] found that Fra-1 is required for the motility and invasiveness of colon carcinoma cells, whereas it had no effect on cell proliferation. Upon Fra-1 silencing, an absence of extending protrusions was observed associated with an increase in stress fibers and focal adhesions, leading to reduced motility of the cells. Based on their experiments, the authors propose that cell motility is promoted by Fra-1 through inactivation of β1-integrin which keeps RhoA activity low.

An initial event in human colorectal carcinogenesis is mutation of the adenomatous polyposis coli protein (APC) which, in its wild-type form, binds to the cell adhesion molecule β-catenin and triggers its rapid degradation. In APC −/− cells, β-catenin accumulates in the cytoplasm, and binds to the T cell-factor/lymphoid-enhancer-factor (Tcf/Lef) transcription factor complex which is then shuttled to the nucleus. *In vitro* studies have shown that one of the target genes of the β-catenin/TCF signalling pathway in colon carcinoma cells is Fra-1 [67]. In a Western blot and immunohistochemical analysis of 12 clinical adenocarcinomas, upregulation of

Fra-1 and β-catenin compared to normal mucosa was shown in every tumour [68]. The authors conclude that Fra-1 overexpression in colon cancer is the result of abnormal nuclear β-catenin expression.

2.9. Skin tumours

The expression pattern of AP-1 family members in normal squamous epithelia suggests that the single Jun and Fos proteins have specific functions in the differentiation process of the skin [69]. In the basal layer of the human epidermis, Fra-1 is the predominant Fos family member. In the stratum spinosum, Fra-1, FosB and c-Fos are detectable, while in the stratum granulosum, c-Fos and Fra-2 are the main Fos proteins. These observations go along with differences in the expression of cytokeratins or other differentiation markers including profilaggrin and involucrin [70].

With respect to the role of Fos family members in skin carcinogenesis, the first interesting experimental results were obtained from the mouse multistep carcinogenesis model, which includes tumour initiation (i.e., by mutagens), tumour promotion (i.e., by TPA) leading to benign skin papillomas, progression to malignant carcinomas, and epithelial-mesenchymal transition resulting in spindle cell tumours. By analysis of this series of events in wildtype and c-Fos null mice, Saez [71] could demonstrate that c-Fos is not required for early stages of carcinogenesis. Yet, papillomas of c-Fos-deficient mice evolve into elongated tumours with hyperkeratinisation and fail to undergo malignant progression. In contrast to ''normal'' papillomas, these horny tumours are deficient in MMP1 and stromelysin, and showed strongly reduced VEGF expression levels. Thus, the failure to progress to invasive skin carcinomas in c-Fos-deficient mice might be due to defects in the degradation of the extracelluler matrix (ECM) as a prerequisite of invasion and to reduced vascularisation.

An additional hint that overexpression of c-Fos might be involved in skin carcinogenesis came from studies with UVA-irradiated keratinocyte human cell lines, which, via activation of the MAP kinases p38 and JNK, show enhanced AP-1 activity and c-Fos expression [72]. Moreover, transgenic mice showing high c-Fos expression in various organs were more susceptible to chemical induction of skin carcinomas after application of 9,10-dimethyl-benzanthracene (DMBA) compared to control animals [73].

In another study, Zoumpourlis [74] analysed the expression of Fos proteins in a series of cell lines corresponding to the different steps in the described mouse model. In comparison to the immortalised keratinocytes, malignant cell lines expressed more and hyperphosphorylated Fra-1 and higher levels of c-Fos and Fra-2. In EMSA experiments, Fra-1 and Fra-2 were the main Jun partners in transformed cells.

A similar series of human cell lines (human epidermal keratinocytes, cells derived from a premalignant lesion and SCC cell lines) was grown in organotypic raft culture followed by RNA isolation and cDNA microarrays [75]. In neoplastic cells, Fra-1 was upregulated about 5-fold compared with normal keratinocytes, accompanied by a switch from cytoplasmic to nuclear localisation. No significant upregulation was observed for c-Fos and Fra-2. Overexpression of Fra-1 led to a reduced AP-1 activity as measured in reporter assays. These results support a model in which c-Fos is necessary for the first steps in carcinogenesis, but Fra-1 promotes full transformation to an invasive phenotype.

2.10. Melanomas

In normal melanocytes, the major proteins involved in AP-1 binding are c-Jun, JunD and FosB [76]. In contrast, radial growth phase-like melanoma cells expressed c-Jun, JunD and Fra-1, and in vertical growth phase-like melanoma cells, only JunD binding was detected by supershifts. In metastatic cell lines derived from patients, one of the two patterns observed in melanoma cell lines was present. One of these metastatic lines was treated with Resveratrol, a polyphenolic compound and antioxidant naturally found in red wine [77] which is a potent tumour preventive agent *in vitro* and in animal models. In c83-2c cells, Resveratrol induced a reversal of malignant properties with morphological changes, reduced anchorage-independent growth and decreased AP-1 binding. Moreover, MHC I antigen and Fas/CD95, which are a prerequisite of effective tumour cell destruction by cytotoxic T lymphocytes, were upregulated in the treated cells. This coincided with increased Fra-1 and Fra-2 expression, and supershift assays revealed a shift of AP-1 binding proteins from c-Jun/JunD/Fra-1 to JunD/Fra-1/Fra-2. After Fra-2 overexpression by transient transfection of melanoma cells AP-1 activity was reduced and MHC class I antigen and Fas protein levels were elevated, which renders the cells more susceptible to immune response. The authors conclude that Fra-2-containing AP-1 dimers might activate a specific set of AP-1 target genes which are involved in the differentiation of melanoma cells.

2.11. Thyroid carcinomas

The role of AP-1 proteins in tumours of the thyroid gland was first investigated in the rat cell system [78]. Comparison of normal thyroid cells with cells expressing either the v-mos or the Ki-ras oncogene showed that in the transformed cells, AP-1 binding and the stability of AP-1 complexes were strongly increased. In normal thyroid cells these complexes consisted mainly of JunD heterodimers, whereas in v-mos-transformed cells heterodimers of Fra-1 with any of the Jun proteins predominated, and

Ki-ras-transformed cells contained Fra-1 and FosB-containing heterodimers. This change is dependent on expression of HMGI-C, an architectural component of the transcription apparatus, and can be partly reverted by antisense HMGI-C expression. The inhibition of Fra-1 expression in transformed cells by stable transfection with antisense vectors resulted in reduced anchorage-independent growth; partial reversion of the morphological changes associated with the transformed phenotype; and reduced expression of the AP-1 target genes MMP1, MMP3 and VEGF.

Changes in c-Fos and Fra-2 expression were not observed in this experimental system. Even a reduced c-Fos expression in malignant papillary carcinomas in comparison with benign human thyroid tissue was observed by Liu [79]. Fra-1 expression was studied by immunohistochemistry in 186 thyroid tissue samples [80]. Fra-1 protein and mRNA was undetectable in normal tissues, but abundant in 100% of the carcinoma samples. In adenomas (88%) and goiters (36%), moderate Fra-1 expression in some of the cases was detected. In conclusion, Fra-1 activation appears to be an early event in thyroid carcinogenesis. If it is also a useful tool in the diagnosis of thyroid tumours, is still a matter of controversy [79,81].

2.12. Esophageal cancer

In an immunohistochemical and *in situ* hybridisation study on c-Fos expression in esophageal tissue samples, c-Fos positivity was detected in 66% of the dysplasias and 53% of the squamous cell carcinomas (ESCC), but in less than 5% of the normal esophageal epithelia [82]. Positive immunostaining was predominantly found in well or moderately differentiated tumours, whereas poorly differentiated carcinomas were mainly c-Fos-negative. These results suggest that c-Fos upregulation might be an early event in esophageal carcinogenesis. In a cDNA array analysis of two ESCC cell lines and one morphologically normal esophageal tissue specimen, 53 genes were found to be upregulated in the cancer cell lines, among them JunD and Fra-1 [83]. Fra-1 overexpression was validated by RT-PCR and further analysed by immunohistochemistry in 61 ESCC tumours: 87% of these cases displayed enhanced Fra-1 expression, in contrast to only weak and focal expression in basal cells of normal epithelia. Similar to c-Fos, Fra-1 overexpression was more often found in well differentiated than in poorly differentiated tumours.

2.13. Hepatocellular carcinomas

In hepatocellular carcinomas (HCC), some experimental data are in favor of a tumour-suppressive function of c-Fos [84]. In murine hepatocytes with conditional c-Fos expression, induction results in morphological changes leading to depolarisation, inhibition of proliferation and finally apoptosis. This effect was even enhanced by oncogenic H-ras expression. In contrast, nodularin, a cyanobacerial toxin acting as tumour promoter in rat liver, upregulates the mRNA expression of c-Fos, FosB and Fra-1 [85]. In an immunohistochemical study on 150 human HCC samples, c-Fos expression was significantly higher in tumours than in non-tumour tissue, whereas no differences in c-Jun expression were found [86]. Targeted overexpression of EGF in transgenic mice leads to the development of HCC after 6–8 months. When the normal liver tissue of these transgenic animals before carcinogenesis was compared to control liver by oligonucleotide microarrays, an overexpression of c-Fos was detected, that might suggest a tumour-predisposing effect of this gene in liver tissues [87].

2.14. Other tumours

One of the etiological agents for gastric cancer is *Helicobacter pylori*. Exposure of gastric cells to virulent strains of this bacterium results in increased AP-1 binding in EMSA experiments. The only Fos protein in these complexes was c-Fos, which was strongly induced on a transcriptional level [88]. Moreover, *H. pylori* activates the MAP kinase cascade which results in phosphorylation of Elk-1 and increased Fos expression.

Among the lymphoma types, a strong overexpression of c-Fos was detected by cDNA array analysis in splenic marginal zone lymphomas (SMZL) [89]. The human T-cell leukemia virus type 1 gene Tax1 stimulates the expression of several cellular genes, among them Fra-1 which is activated in a similar way as induced by serum [90].

In prostate cancer, the mechanisms underlying the development of advanced, androgen-insensitive tumours from the initial androgen-dependent carcinomas are still poorly understood. In an IHC study, the expression of c-jun, phosphorylated c-jun and c-Fos was analysed in tumours before and after the onset of androgen-independence [91]. No significant differences in expression levels of these factors were observed. Hormone-refractory prostate tumours are characterised by an overexpression of interleukin (IL)-6. In an attempt to investigate the mechanisms underlying this upregulation in prostate carcinoma cell lines, Zerbini found that IL-6 promoter activation results from combined activation of NFkB, JunD and Fra-1 [92]. In an androgen-sensitive cell line, no Fra-1 binding to the IL-6 promoter was observed. This indicates that Fra-1 upregulation might be involved in progression of prostate carcinomas.

Deregulated Fra-1 expression was also found by cDNA arrays in a highly metastatic rat pancreatic carcinoma cell line compared to its non-metastatic counterpart [93]. In contrast, increased cell motility of a hamster pancreatic carcinoma cell line was accompanied

by a high induction of c-Fos, which turned out to be a necessary step in the development of cell motility in antisense experiments [94]. In human pancreatic tumours, c-Fos mRNA and protein overexpression was found in the majority of the carcinomas [95,96].

In squamous cell carcinomas of the head and neck (HNSCC), studies focusing on c-Fos expression in tumour tissues do not indicate a role of this oncogene in tumour development and progression: in a Northern blot study, no difference in c-Fos mRNA expression between clinical carcinoma tissue and matched normal mucosa was detected [97]. Immunohistochemically, c-Fos expression in differentiated oral carcinomas was found to be stronger than in undifferentiated tumours [98]. Yet, experimental data indicate that Fra-1 might be involved in HNSCC carcinogenesis, since cytokine expression which is characteristic of these tumour cells is upregulated by AP-1 complexes including Fra-1 [99].

In a cDNA microarray analysis of various types of salivary gland tumours, one of the genes whose overexpression characterised pleomorphic adenomas in comparison to normal glands was Fra-2 [100]. The gene expression profile in non-malignant and malignant nasopharyngeal cells was also studied using microarrays hybridised to cDNA prepared from pooled mRNA derived from 7 carcinomas cell lines and 5 normal cell lines. Thirteen genes were over-expressed in malignant NPE cells, among them Fra-1 with a nearly 10-fold increased mRNA level [101]. The differential expression was confirmed by RT-PCR.

3. Discussion and perspectives

The data presented above support the model that a shift in the expression in AP-1 transcription factors, namely Fos family members, is an important step in carcinogenesis and/or progression. Early studies suggested that Fra-1 and Fra-2, due to their lack of a trans-activating domain which is characteristic of c-Fos and FosB, might exert inhibitory functions on tumour cell growth. Yet, recent data point to a positive effect of Fra-1, and partly Fra-2, on tumour invasion and progression in many tumour types (except cervical cancer; Table 1). In order to test whether Fra-1 can take over functions of c-Fos under physiological conditions, knock-in mice were generated in which a deleted *c-fos* gene was replaced by *fosl1 (fra-1)* [102]. Interestingly, the phenotypic defects of c-Fos null mice including growth retardation, impaired tooth development and osteopetrosis were rescued by Fra-1 overexpression, although Fra-1 failed to induce expression of c-Fos target genes (MMP13, vimentin) in mouse fibroblasts. This is in accordance with the results on various cancer cell types where Fra-1 alters the biological behavior of the cells without direct activation of

Table 1
Role of Fra-1 in experimental systems and clinical tumour samples

Tumour type	Role of Fra-1	References
Breast cancer	Enhanced motility and invasion, morphological changes, transcriptional activation of uPA, PAI-1, uPAR, osteopontin, HMGI(Y), MMP's, VEGF, etc., downregulation of β1-integrin; in clinical tumours correlation with an undifferentiated, ER-negative phenotype	[21–28,30–32]
Cervical carcinoma	Suppression of tumourigenicity; in clinical samples downregulation in carcinomas in comparison to early precancer lesions	[42–46]
Ovarian carcinoma	Enhanced motility; overexpression in ras-transformed ROSE cells	[51,52]
Mesotheliomas	Morphological changes, increased colony formation in soft agar, increased expression in asbestos-treated cells; upregulation of CD44 and c-met	[53,54]
Lung cancer	Increased expression in bronchial epithelial cells after exposure to environmental toxicants; involved in transformation from SCLC to NSCLC	[55,56,62]
Colorectal cancer	Enhanced motility and invasion, morphological changes, inactivation of β1-integrin	[29,63,65]
Skin SCC	Increased expression in transformed, malignant cells	[71,72]
Thyroid carcinomas	Up-regulation in ras- or mos-transformed thyroid cells, morphological changes, anchorage-independent growth, upregulation of MMP1, MMP3, VEGF; in clinical samples upregulation of Fra-1 in malignant tumours	[75,78]
Esophageal cancer	Up-regulated in cancer cell lines vs. normal cells; enhanced expression in clinical carcinomas Vs. normal esophageal tissue	[80]
Prostate cancer	Probably involved in progression to hormone-independence	[89]
Pancreatic cancer	Up-regulated in highly metastatic cells	[90]
Nasopharyngeal carcinoma	Overexpressed in malignant nasopharyngeal cells	[98]

AP-1-responsive promoters (see above). Therefore, 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 [102].

Fra-1 might also be a valuable target for therapy. Some tumour-preventing agents function by deregulation of Fra-1 expression in model systems, i.e., resveratol [77], green tea [103] and curcumin [49]. In a mouse model system, Fra-1 even proved suitable for vaccination against growth of mouse breast cancer cells [104]: attenuated *Salmonella typhimurium* strains transformed with plasmids encoding polyubiquitinated Fra-1 and IL-18, an enhancer of cellular immune reactions, resulted in marked inhibition of tumour growth and metastases in BALB/c animals, accompanied by activation of T-cells and natural killer cells and impaired angiogenesis.

Yet, most data concerning the function of Fra-1 in carcinogenesis are based on experimental results, and the function of these transcription factors in clinical tumours is still poorly understood. In most of the tumour tissues analysed so far, Fra-1 expression is far below the protein amounts found in undifferentiated cell lines, and the electrophoretic mobility of the Fra-1 protein indicates that it is not highly phosphorylated which leads to its stabilisation and activation *in vitro*. If the small Fra-1 amounts in tumours have a similar effect to that seen in experimental systems, or if Fra-1 expression in single cells or cell clones within the tumours contributes to local invasion and metastasis, should be further analysed.

In contrast to the bulk of data on the function of c-Fos and Fra-1, far less is known about the role of FosB and its smaller splice variants FosB2 and deltaFosB2, and Fra-2, which is often expressed more strongly than Fra-1 in clinical cancer tissues. The further study of the role of all Fos proteins in carcinogenesis, especially *in vivo*, will be of great importance and will probably open new perspectives for therapy.

Acknowledgements

I apologise to those people whose work was not cited in this review due to space limitations. I thank Ana-Maria Bamberger and Thomas Löning for helpful discussions and critically reading the manuscript.

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