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Transcriptional regulation of cell invasion: AP-1 regulation of a multigenic invasion programme

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Abstract

The focus of this review will be on the regulation of the multigenic invasion programme by activator protein-1 (AP-1). Investigation of AP-1-regulated gene expression in transformed cells can be used to identify the genes in the multigenic invasion programme and to validate them as targets for diagnosis or therapy.

Keywords: AP-1; Invasion; v-*fos*

1. Introduction

Tumour cell invasion of surrounding tissue makes the pathological distinction between benign and malignant tumours, and contributes to their metastatic potential [1]. Thus, therapies designed to inhibit the conversion of a benign to a malignant tumour might be useful strategies for the treatment of cancer. The difficulty previously has been the identification of targets specific for the invasive phenotype with the important exception of extracellular proteases and their inhibitors, which serve as prototypes for the validation of invasion targets [2,3]. The application of expression genetics [4] to the analysis of cancer has made the identification of the differences in gene expression responsible for invasion, and perhaps metastasis, a realistic possibility. Indeed, there are currently many studies underway using a variety of technologies designed to determine gene expression profiles that will define the cancer cell or transformed phenotype. Cancer gene expression profiles consist of both upregulated and downregulated genes. The upregulated genes encode proteins that are potential enhancers of the transformed phenotype, while the downregulated genes encode proteins that are potential suppressors of the transformed phenotype [4]. Among the differentially expressed genes will be those responsible for the processes of invasion and metastasis. The challenge will be to identify those genes that confer invasiveness and metastatic potential upon the tumour from the myriad of differentially expressed genes.

2. Invasion is a regulated process

Invasion is a complex multistage process [1]. In some cases invasion may result from a co-ordinated interaction between the tumour, stroma and infiltrating inflammatory cells [2,3]. The contribution of the non-tumour cells to invasion may be through the paracrine production of cytokines, growth factors and extracellular proteases which enhance tumour cell invasion. The non-tumour cells may also produce suppressors of invasion, such as inhibitors of extracellular proteases, which the tumour cells must overcome. For invasion to occur the net effect of the multicellular interaction must favour invasion. The invasive cell must alter its cell to cell and cell to extracellular matrix (ECM) adhesions, suppress anoikis, control degradation of the ECM to facilitate movement through it, and reorganise its cytoskeleton to support cell motility [1].

Invasion is a process used by many types of normal cells, when it is necessary for them to migrate through tissue boundaries. For normal cells, invasion, like proliferation, is a tightly regulated process that ceases when the stimulus is withdrawn [1]. This implies that there is a regulated programme for invasion, similar to that for

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the cell cycle that can be activated by extracellular signals. It is well established that growth factors, among other signals, can activate the invasion programme in some cell types, as well as stimulating progression through the cell cycle [5–10]. The complexity of the invasion process and its tight control in normal cells suggests that it is a transcriptionally-regulated multigenic programme [5,7,11].

The growth factor-ras-raf-mek-erk signal transduction pathway is frequently activated by mutations in its component proteins in many types of cancers [12]. This results in its sustained stimulation, which leads to the changes in transcription factor activity [13–15] that are required for activation of the invasion programme. Among the transcription factors which function downstream of the growth factor ras signal transduction pathway are activator protein (AP)-1 [3,5,16], Ets [17–19] and Nuclear factor kappa B (NFkB) [20,21]. Each of these has been implicated in the regulation of genes involved in invasion.

3. AP-1 and invasion

AP-1 is a complex of transcription factors comprising heterodimers of *Fos*- and *Jun*-family proteins that bind to a consensus DNA sequence usually in the promoter region of genes [22] (Fig. 1). The prototypes of each family were first identified as the cellular homologues, c-*fos* and c-*jun*, of retroviral oncogenes, v-*fos* [23] and v-*jun* [24], highlighting the importance of AP-1 in transformation. Transformation by oncogenes that function in the growth factor signal transduction pathway, such as ras [13], raf [15] and mek [14], results in a dramatic increase in AP-1 component protein expression and is dependent upon the resultant increase in AP-1 activity [6,25–31]. AP-1 activity is also required for squamous cell carcinoma formation in transgenic mouse model systems [25,27] and the growth of human and mouse tumour-derived cell lines in animals [32].

The concept that AP-1-regulated genes play a role in the invasive process is based on several observations. Firstly, inducible matrix metalloproteinases (MMP) share a consensus AP-1 binding site at position-66 to-72 in their promoters, which is responsible for basal expression and responsiveness to external stimuli such as growth factors [3]. Many invasive tumours express high levels of MMPs and inhibition of MMP activity results in reduced invasion. Not only do MMPs play an important role in the degradation of ECM, they also have a role in cell motility, another important aspect of invasion [33]. MMPs are also associated with metastasis as they are essential components in the process of angiogenesis [34] and tumour cell intravasation [35]. There is *in vivo* evidence that AP-1 regulates the expression of MMPs. The induced overexpression of c-*Fos* in mice where the c-*Fos* transgene is under the control of interferon results in an increase in MMP13 in specific tissues [36]. In c-*fos* nullizygous mice, in which the progression from papilloma to carcinoma is inhibited, induction of *MMP3* and *MMP13* gene expression by peptide growth factors is also inhibited [27]. The regulation of MMP expression by AP-1 in tumours indicates that AP-1 might play a direct role in tumour cell invasion.

Secondly, altering the expression of AP-1 component proteins also affects cellular invasion. Increased expression of AP-1 component proteins and AP-1 activity enhances invasion [5,37,38]. Cells transformed by oncogenic forms of *Fos* or *Jun* proteins are invasive [5,7]. Normal rat fibroblasts (208F cells) are non-invasive, but can be induced to invade by exposure to growth factors such as epidermal growth factor (EGF) or platelet-derived growth factor (PDGF) [5]. This results in an increase in AP-1 component protein expression with a dramatic increase in *Fra*-1, c-*jun* and *jun*D expression (Fig. 2).

Inhibition of the expression of AP-1 component proteins and AP-1 activity reduces the invasiveness of cells. Inhibition of AP-1 activity by the expression of the dominant negative mutant of c-*jun*, TAM67, reduces the invasiveness of a variety of cell types including murine and human squamous cell carcinomas [6,16,32] and rat fibroblasts [7]. The invasiveness of *fos*-transformed and growth factor-stimulated rat fibroblasts is also inhibited by antisense oligonucleotides to AP-1

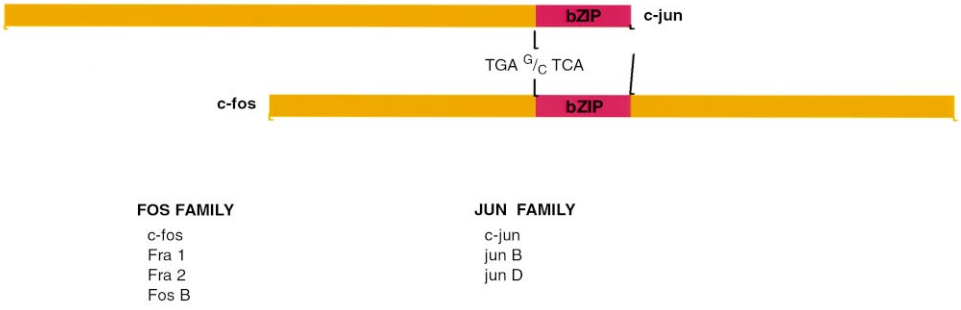

Fig. 1. The AP-1 transcription factor.

component genes [7]. The expression of TAM67 as a transgene in the mouse skin inhibits tumour formation [25].

Transformation by v-*fos* activates the multigenic invasion programme without stimulating cell cycle progression [5]. This separates the proliferative component of transformation from morphological transformation, which is associated with invasion [5,39]. Mutational analysis of the fos oncoproteins demonstrates that their transforming activity is dependent upon their ability to form complexes with jun family proteins, and to transactivate gene expression [22]. This indicates that fos oncoproteins transform cells through changes in gene expression [5,7,11,40]. In this regard, analysis of changes in gene expression upon fos transformation of cells should identify genes, which comprise the multigenic invasion programme (data not shown, [5]). One of the earliest changes in gene expression upon fos-transformation is the increase in expression of fra1, a fos family member which has transforming activity [39,41–43]. This suggested that v-*fos* may alter the expression of the other AP-1 component proteins, such that they reflect the changes in AP-1 component protein expression induced by transformation with *ras* [13], raf [15] or mek [14] that are important for transformation. We have observed a similar increase in the expression of AP-1 component protein expression in *fos*-transformed cells (Fig. 2). This is important point as it indicates that the AP-1-directed changes in gene expression in fos-transformed cells reflect those changes seen in cells transformed by oncogenes that function in the growth factor signal transduction pathway.

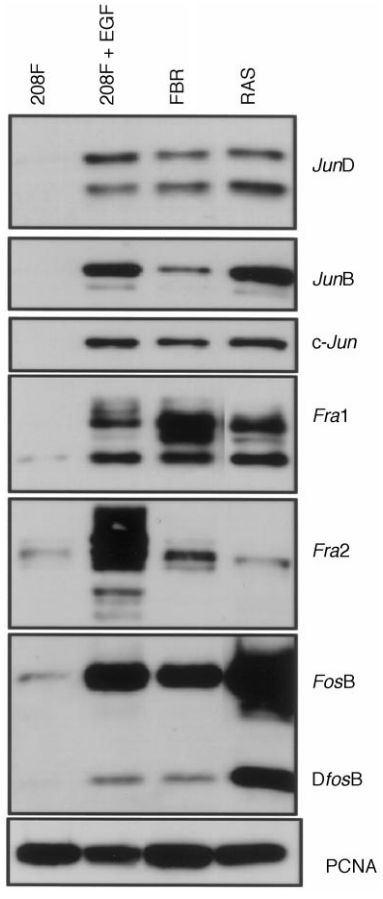

Fig. 2. Western blots for AP-1 component proteins in nuclear extracts from 208F cells, 208F cells plus EGF, and 208F cells transformed by FBR v-*fos* or v-*ras*. The blot was probed with antibodies to proliferating cell nuclear antigen (PCNA) to demonstrate equal loading of the nuclear extracts. .

4. Identification of differentially expressed genes

The technology for identification of differentially expressed genes has been revolutionised over the past 10 years. There are basically four techniques; construction of subtracted cDNA libraries [44], differential display [45], reverse transcriptase representational difference analysis [46], serial analysis of gene expression and differential screening of DNA microarrays [47]. With the exception of the DNA microarray method, the identity of each differentially expressed gene must be determined by nucleic acid sequence analysis and database comparisons. If the differentially expressed gene represents a known function its contribution to the transformed phenotype may become apparent. The validation of the differentially expressed genes, as potential targets for diagnosis and/or treatment, depends upon the demonstration that its differential expression can be detected in actual tumours and that its altered expression affects the tumorigenic process in a functional assay. Both of these conditions have been met for AP-1-regulated MMPs [3].

We have used a polymerase chain reaction (PCR)-based protocol [44] to create subtracted cDNAs specific for genes differentially expressed (up- or downregulated) in *fos*-transformed fibroblasts, as a means of identifying the genes that make up the multigenic invasion programme. We have identified over 200 genes in each library. Northern analysis of randomly selected clones indicated that 84% of the upregulated cDNAs are also upregulated by *ras* transformation and 57% are upregulated in normal fibroblasts treated with EGF to become invasive. This suggests that the targets of AP-1 in fos-transformed cells are also targets in cells transformed by sustained activation of the growth factor signal transduction pathway. Eighty-six per cent of the genes in the upregulated library are downregulated to some degree by expression of the dominant negative mutant of c-*jun*, TAM67. This indicates that most of the upregulated genes are, either directly or indirectly, under the control of AP-1. For the downregulated genes, 69% and 56%, respectively, are also downregulated in *ras*-transformed and growth-factor-treated cells. However, only 30% of the cDNAs show any

increase in expression due to TAM67. The expression data suggest that most of the cDNAs in each library are differentially expressed as a consequence of transformation. The strong differential expression of most of these genes at the RNA level suggests that they may be reasonable diagnostic targets. Further validation of their differential expression in tumours and their relationship to prognostic factors must be established.

Sequence analysis and database searches of randomly selected clones reveals the identity of approximately 70% of the cDNAs in each library, while 28% are detected as expressed sequence tags (ESTs), but to which no function can as yet be assigned. The functions of the identifiable genes supports the contention that AP-1 in the context of transformed cells regulates a multigenic invasion programme. Over half of the upregulated genes encode proteins which are associated with invasion, such as extracellular proteases, cell-to-cell adhesion molecules, cellular receptors for ECM components, cytoskeletal regulatory proteins, motility factors, apoptosis inhibitors, angiogenesis and transcription regulators (Table 1). The downregulated genes

Table 1
Upregulated genes

Gene product	Function
MMP10	Protease
Urokinase	Protease
MMP9	Protease
MMP2	Protease
Cathepsin L	Protease
Cathepsin B	Protease
Cathepsin D	Protease
Plasminogen activator inhibitor (PAI)-1	Protease inhibitor
Mts1/9Ka	S-100Ca + binding protein
p11 Calpactin	S-100Ca + binding protein
Oncomodulin	S-100Ca + binding protein
p67 LBP	Laminin binding protein
p35 Mac-2/gelectin-3	Laminin binding protein
CD44	Hyaluronan receptor
Transforming growth factor (TGF)β-induced protein	Cell adhesion
Ezrin	Actin/membrane binding protein
Vimentin	Adhesion/cytoskeleton
Arf-1	Actin cytoskeleton regulator
Scar2	WASP family ARP2/3 regulation
Arp2/3 p16 subunit	Actin cytoskeleton regulator
Rho kinase α	Rho-dependent kinase
GAK	Cyclin G-associated kinase
Krp 1	Kelch-related protein
Cyclooxygenase (Cox)-2	Cyclooxygenase-2
Tyrosine phosphatase	Protein tyrosine phosphatase
Protein phosphatase (PP)2A B subunit	PP2A regulation
N-acetyl transferase (NAT)1	Translation repressor
Histone deacetylase (HDA)C4	Histone deacetylase
Metastases-associated (MTA)1	Histone deacetylase subunit
RbAp46	Histone deacetylase subunit
SAP18	Histone deacetylase subunit
DNA methyltransferase (Dnmt)1	DNA methyltransferase
Tip30	Transcription factor
*Fra*1	Transcription factor
*Fra*2	Transcription factor
c-*jun*	Transcription factor
*jun*D	Transcription factor
Vascular endothelial growth factor (VEGF)	Angiogenesis
Angiogenin	Angiogenesis
Clusterin	Apoptosis
NAPOR	Apoptosis

Mts, metastasis related protein; LBP, laminin binding protein; Arf, ADP-ribosylation factor; WASP, Wiscott-Aldrich syndrome protein; Arp, actin-related protein; GAK, cyclin G-associated kinase; Krp, kelch related protein; NAT, novel translational repressor; RbAp46, Rb-associated protein; SAP18, mSin3-associated polypeptides; Dnmt, DNA 5-methylcytosine transferase; Fra, fos related antigen; NAPOR, neuroblastoma apoptosis-related RNA binding protein.

Table 2
Downregulated genes

Gene product	Function
Lysyl oxidase	Collagen cross-linking
Fibronectin	Extracellular matrix
Collagen type 1	Extracellular matrix
Tenascin-X	Adhesion/cell migration
MGC-24	Adhesion
TSC-1	Adhesion/tumour suppressor
Fibrillin-1	Extracellular matrix
β-Actin	Cytoskeleton
Tubulin beta-5	Microtubules
TSC-36/FRP	Follistatin-related protein (FRP)
PKC-ξ binding protein	PKC regulation
TGF-β binding protein	TGF-β regulation
Protocadherin 43	Cell–cell interaction
Frizzled related protein	Wnt signal antagonist
Sprouty	Fibroblast growth factor (FGF) antagonist
FISP-12	ECM signalling
Fez	Protein kinase C (PKC)-ζ regulation
PP2A B subunit	Protein phosphatase (PP)2A targeting
Necdin	Growth suppressor
Caspase 11	Interleukin-converting enzyme (ICE) complexes
Gas-5	RNA processing
RNA cleavage factor	RNA processing
Transcription factor S-II	RNA elongation
IRF-1	Transcription factor
Cellular retinol-binding protein (CRBP)1	Transcription factor
STAT6	Transcription factor
c-*fos*	Transcription factor

MGC, mucin-like glycoprotein; TSC-1, tuberosclerosis complex; TSC-36/FRP, TGF-beta1-stimulated clone/follistatin related protein; TGF, transforming growth factor; ECM, extracellular matrix; ICE, interleukin converting enzyme; GAS, growth arrest specific; IRF, interferon regulatory transcription factor; STAT, signal transducer and activator of transcription.

include tumour suppressors, protease inhibitors, ECM component proteins, cell-to-cell and cell-to-ECM adhesion proteins, negative regulators of ras, wnt and transforming growth factor (TGF)β signalling pathways and transcription factors (Table 2).

5. Functional analysis of differentially expressed genes

5.1. Upregulated gene expression is required for invasion

For a differentially expressed gene to be considered a component of the invasion programme, its differential expression must impact upon the process of invasion. For upregulated genes the expression or function of the protein must be inhibited, and that inhibition must serve to reduce invasion or some aspect of the invasion process [7,11,40]. This has been accomplished for several of the MMPs [2,3]. Inhibition of expression or pharmacological inhibition of protease activity prevents invasion in *in vitro* invasion assays and tumour assays. The expression of specific inhibitors, tissue inhibitors of MMPs (TIMP)s, of MMPs by the tumour cells also inhibits invasion and tumour formation.

Although AP-1 regulates the expression of proteases that are important for invasion, other upregulated genes identified clearly demonstrate that they function as part of a co-ordinated invasion programme, which is multigenic. Cell determinant CD44 is a type-1 cell surface receptor for hyaluronan that has been implicated in tumour cell invasion and metastasis [48] and which contains a consensus AP-1 binding site in its promoter [49]. CD44 expression is upregulated in v-fos oncoprotein-transformed cells and in rat fibroblasts stimulated by EGF [7]. In both cases, inhibition of AP-1 activity reduces the expression of CD44. An 80% reduction of CD44 expression by antisense oligonucleotides significantly inhibits invasion by fos-transformed or growth factor-stimulated fibroblasts. The demonstration that a reduction in the expression of CD44 inhibits invasion indicates that the invasion programme must be finely balanced and regulated for efficient invasion. Expression of CD44 cDNA in normal cells does not render the cells invasive [7]. This also suggests that the complete programme must be expressed for the cells to become invasive. This is an important concept in the design of anti-invasion therapeutics. On one hand, it suggests that inhibition of AP-1 activity may be an effective anti-invasion therapy and on the other hand, that targeting the components of the invasion programme may provide many potential targets, making the selection of resistant cells less likely.

The concept that invasion results as a consequence of activation of a multigenic functionally co-ordinated programme is suggested by the identification of upregulated genes which functionally, if not physically, interact. One of the genes identified as being upregulated in fos-transformed cells is *ezrin* [50,7]. Ezrin is a member of the ERM family of proteins, which regulate interactions between membrane proteins and the actin cytoskeleton [51–53]. Ezrin in normal cells exists primarily as a cytoplasmic protein with latent actin binding potential. Growth factor stimulation leads to the phosphorylation of ezrin that unmasks its actin-binding activity, and results in its translocation to sites of membrane ruffling where it interacts with the intracellular domain of CD44. CD44 and ezrin co-localise in the extending pseudopod of fos-transformed cells [7,40]. Specific ablation of ezrin in the extending pseudopod of fos-transformed cells results in pseudopod retraction and inhibits cell motility, which is required for invasion [40]. As with CD44, expression of ezrin in normal cells does not render them invasive. Thus, this also demonstrates that decreased function of a single component of the invasion programme is sufficient to inhibit an important aspect of invasion, but the expression of a single component is insufficient to render the cells invasive.

Another component of the invasion programme MMP9 has been shown to interact with the extracellular domain of CD44 [54,55]. This interaction concentrates MMP9 at the sites of cell extension where it controls cells migration and/or degradation of the cell–ECM interactions necessary for cell motility through the ECM. This study demonstrated, at the functional level at least, one of the roles of CD44 in the invasion process and further supports the concept of invasion resulting from a co-ordinated programme.

The concept of a multigenic invasion programme is further strengthened by functional analysis of a novel gene. Sequence analysis indicated that one of the upregulated genes in *fos*-transformed cells is a new member of the kelch family of proteins, kelch-related protein 1 (Krp1) [11]. Kelch family proteins are defined by the presence of a series of repeats of 50 amino acids punctuated by di-glycine residues [56]. Krp1 is a member of the subfamily of kelch proteins that have a POZ protein interaction domain adjacent to the amino-terminus, and interact with actin, or components of actin-containing structures. Analysis of Krp1 revealed that it co-localises with actin at the tips of extending pseudopodia in close proximity to ezrin and CD44-containing membrane ruffles, although most of Krp1 in transformed cells remains in the cytoplasm. Increased expression of Krp1 in v-*fos*, or v-*ras*-transformed cells results in dramatically elongated pseudopodia. Expression of either kelch or the POZ domain alone results in pseudopod truncation and an inhibition of cell motility, similar to that observed upon ablation of ezrin. Krp1 expressed in normal fibroblasts remains cytoplasmic with no obvious association with actin or membranes. Its intracellular localisation, as well as its expression, appears to be transformation-specific. Treatment of normal cells

expressing exogenous Krp1 with sufficient EGF to induce morphological transformation and invasion results in its translocation to actin-containing membrane structures after 4 h and eventually to the tips of extending pseudopodia [11]. The delay in its translocation from the cytoplasm suggests that it needs other components of the invasion programme, which regulate its localisation and thus control its activation. The role that Krp1 plays in pseudopod elongation and motility suggests that it is functioning in concert with ezrin, CD44 and MMP9.

It is obvious from the list of upregulated genes that not all have a function that would directly affect pseudopod elongation and cell motility. Three of the genes are components of histone deacetylase complexes, Sap18, RbAp46, MTA1 [57], and one is DNA 5′-methyltransferase, dnmt1 [39]. DNA methylation and histone deacetylation are associated with a suppression of gene expression [58–60], and may help to explain the large number of genes found to be downregulated in transformed fibroblasts and tumours. Indeed, inhibition of *dnmt1* expression, or treatment of cells with an inhibitor of histone deacetylase activity, trichostatin A (TSA), inhibits morphological transformation by fos oncoproteins [61]. Recently, it has been demonstrated that dnmt1 can form complexes with histone deacetylases [62] suggesting that they may function co-ordinately in the regulation of gene expression necessary for morphological transformation and invasion. We have implicated histone deacetylase as a component of the invasion programme as we have shown that TSA is a potent inhibitor of invasion in *fos*-transformed and growth factor-stimulated fibroblasts (data not shown).

6. Downregulated gene re-expression inhibits invasion

Just as mutations can inactivate tumour suppressor genes which regulate the cell cycle such as *Rb* and *p53* [12], mutations also inactivate tumour suppressors which function to suppress invasion such as *PTEN* (phosphatase and tensin homologue) [63], *E-cadherin* [64], and *SMAD/DPC4* [65]. As pointed out by Sager [4] there are many more genes downregulated at the level of gene expression in tumours than are mutationally inactivated. It has been suggested that tumour suppressor gene expression may be repressed by methylation and histone deacetylation. This would fit with the increased expression of dnmt1 and HDAC (hystone deacetylase) component proteins in fos-transformed cells and the inhibition of invasion by TSA.

Analysis of the downregulated genes identifies some candidate genes such as protease inhibitors, and ECM components which may function as suppressors of

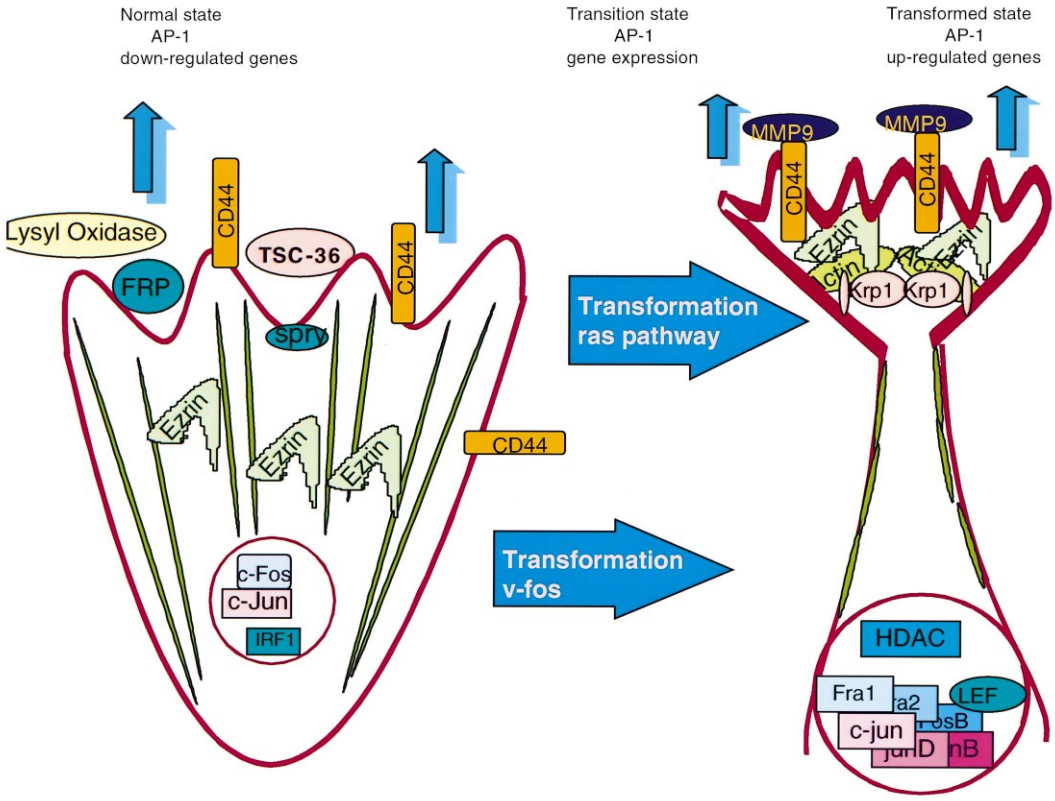

Fig. 3. Model of how AP-1 regulates a multigenic invasion programme.

invasion. To confirm that a downregulated gene represents a *bone fide* invasion suppressor, it is necessary to express the gene in a transformed cell and determine if its expression reduces the invasiveness of the cells. This is the case for known protease inhibitors such as TIMPs [2,3] and the serine protease inhibitor, maspin [66].

Re-expression of other genes identified through their downregulation in tumour cells have also been demonstrated. Drg-1 was isolated due to its downregulation in colon cancers [67]. Treatment of colon cancer-derived cell lines with TSA increases Drg-1 expression suggesting that it is downregulated as a consequence of methylation and deacetylation. Overexpression of Drg-1 in a metastatic colon cancer cell reduced both *in vitro* invasion and metastatic potential.

Another gene identified through its downregulation in prostate cancer, KAI1 [68] upon increased expression in invasive tumour-derived cells, suppresses invasion through inhibiting cell adhesion and motility [69,70].

Of the genes known to be downregulated in fos-transformed cells lysyl oxidase [71,72] and IRF-1 [73,74] are known suppressors of ras transformation . We have demonstrated that increased expression of a target gene of TGF-β signalling, TSC-36 (Mashimo, 1997 #81; Shibanuma, 1993 #80; or follistatin-related protein (FRP) Zwijsen, 1994 #82) in fos-transformed cells inhibits their *in vitro* invasion without affecting growth characteristics. A related molecule, SPARC, shown to be downregulated in v-*jun*-transformed cells, inhibits tumour formation in animals without affecting the growth characteristics in tissue culture [75–78].

These examples indicate that genes downregulated in *fos*-transformed cells may be part of the multigenic invasion programme and that their downregulation is necessary for invasion. The inhibition of invasion by the re-expression of single genes also supports the concept of a multigenic invasion programme.

We propose the following model to explain the AP-1-regulated multigenic invasion programme. In the model cells exist in three states: the normal state; the transition state; and the invasive (transformed) state (Fig. 3).

The normal state is characterised by signalling networks which carry out the normal function of the cells and prevent it from becoming invasive. This state would be maintained at least in part by the expression of genes which are downregulated in the invasive state. The transition state is initiated by strong and sustained activation of the growth factor signal transduction pathway that stimulates the changes in the expression of AP-1 component proteins which increase AP-1 activity and alter the selection of target genes to those expressed in the invasion programme. This can also be initiated directly by induction of v-*fos*. Over time (days) there are changes in gene expression which shift the signalling networks to the invasive state. The changes in gene expression although initiated and maintained by alterations in AP-1 activity do not have to be direct AP-1 target genes in all cases (Fig. 3).

7. Conclusion

Invasion is a complex process, which is regulated, at least in part, by increased activity of the family of transcription factors known as AP-1. Sustained activation of the growth factor *ras* signal transduction pathway results in the increased activity and change in AP-1 composition necessary to target the genes which comprise the multigenic invasion programme. The investigation of *fos*-transformed fibroblasts has allowed us to identify the genes which constitute the invasion programme by identifying the genes that are differentially expressed. The invasion programme consists of both upregulated and downregulated genes. Functional analysis of these genes using assays which measure either complete invasion or the individual processes which contribute to the invasion process support the concept that the differentially expressed genes function in a coordinated manor to enhance invasion. An important point from the functional analyses is that inhibition of a single upregulated gene, or re-expression of a single downregulated gene is sufficient to render the invasion programme ineffective. This emphasises the need to identify as many differentially expressed genes as possible in cancer, as they constitute a large potential pool of targets for the development of cancer diagnostics or therapeutics.

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