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Role of the AP-1 transcription factor FOSL1 in endothelial cells adhesion and migration.

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Abstract

Vasculogenesis and angiogenesis, the fundamental processes by which new blood vessels are formed, involve the proliferation, migration, and remodeling of endothelial cells. Dynamic adhesion of endothelial cells to extracellular matrix plays a fundamental role in all these events. Key regulators of endothelial cells adhesion and migration are the $\alpha v\beta 3$ and uPA-uPAR complexes. The $\alpha v\beta 3$ integrin heterodimer is the receptor for extracellular matrix components such as vitronectin and is overexpressed on the cell surface of angiogenic endothelial cells, but not quiescent cells lining normal vessels. The uPA-uPAR complex contributes to extracellular matrix remodelling by mediating proteolytic activity at the leading edge of migrating cells. We recently reported that the FOSL1 transcription factor of the AP-1 family plays a pivotal role in the regulation of the level of the $\alpha v\beta 3$ and uPA-uPAR complexes on the surface of endothelial cells. In this commentary, we review the current knowledge of αv and $\beta 3$ transcriptional regulation in endothelial cells and discuss the role of FOSL1 in angiogenesis.

Angiogenesis is a crucial process during embryonic development and physiological tissue homeostasis and repair in the adult. On the other hand, pathological angiogenesis is

frequently implicated in diseases, including cancer, rheumatoid arthritis, diabetic retinopathy and age-related macular degeneration. During this process, endothelial cells proliferation and migration lead the sprout of new blood vessels from pre-existing ones and regulation of endothelial cell adhesion to extracellular matrix (ECM) is particularly important. Integrins are heterodimeric transmembrane receptors that bind ECM proteins such as fibronectin, laminins, collagens and vitronectin. Functional integrins are non-covalent heterodimeric complexes composed of paired α and β subunits. In humans, 18 α and 8 β different subunits can combine to form dimers with variable specificity of binding to one or more ECM components.¹ Ligand binding triggers integrins conformational changes and consequent activation of specific cytoplasmic signaling pathways.^{1,2}

Whereas many integrins are ubiquitously expressed in adult tissues, $\alpha v\beta 3$ and $\alpha 5\beta 1$ integrins are expressed at higher levels on the cell surface of activated endothelial cells in physiological and pathological angiogenesis, but not in normal quiescent vessels,^{3,4} similarly to what has been observed for the vascular endothelial growth factor receptor-3 (VEGFR-3)⁵⁻⁷ which is induced in endothelial cells undergoing new vessel sprouting. These features render $\alpha v\beta 3$ and $\alpha 5\beta 1$ integrins promising targets for pharmaceutical inhibition of pathological angiogenesis.

Despite the growing interest for anti-angiogenic therapies based on integrin $\alpha v\beta 3$ antagonism, the molecular mechanisms for the transcriptional regulation of these subunits are not fully understood. The potent angiogenic growth factor bFGF promotes the conversion of endothelium from resting to its active angiogenic state and this action is mediated, at least in part, by the induction of the homeobox transcription factor HOX D3. Boudreau and colleagues demonstrated that HOX D3 binds directly to the promoters of the $\alpha 5$ and $\beta 3$ integrin subunits, inducing their expression.^{8,9} The binding of HOX D3 to target DNA is enhanced by PBX1, a transcription co-factor expressed at higher levels in activated angiogenic endothelial cells and it is required for angiogenesis induced by bFGF.¹⁰ Forkhead transcription factor FOXC2 is another essential regulator of blood and lymphatic vessels formation.^{11,12} In particular, FOXC2 positively regulates endothelial cell migration and adhesion by binding directly to multiple Forkhead-binding elements within two homology regions in the integrin $\beta 3$ promoter and inducing its expression.¹¹

The human promoters of αv and $\beta 3$ subunits contain GGA/TCC repeats and GC rich sequences, which are binding sites for the ubiquitous transcription factor SP1.¹³⁻¹⁵ By chromatin immunoprecipitation (ChIP) we recently demonstrated that SP1 actually binds to both αv and $\beta 3$ promoters.¹⁶

Similarly to other members of the AP-1 transcription factor family, which are required for endothelial cell survival,¹⁷ FOSL1 (FOS-Like1; also named FRA1), was supposed to play a role in angiogenesis because its mouse orthologue Fosl1 has been previously shown to be required for the correct vascularization of extra-embryonic tissues.¹⁸ In a mouse model of embryonic development *in vitro*, we demonstrated that *Fosl1*^{-/-} embryonic stem cells maintain the capacity to differentiate in endothelial cells but fail to correctly assemble and form disorganized vessel-like structures. Human primary umbilical vein endothelial cells (HUVEC) silenced for FOSL1 expression displayed similar features, and they failed to form *in vitro* capillary-like structures. By gene expression profile we found that FOSL1-silenced HUVEC overexpressed the αv and $\beta 3$

integrin subunits. We demonstrated that endogenous FOSL1 binds to the promoters of both integrin subunits repressing their gene expression. This effect is possibly mediated by epigenetic mechanisms, since FOSL1 binding to these promoters induces the deacetylation of histone H4 at lysine 16.

As a consequence of αv and $\beta 3$ overexpression, endothelial cells knockdown for FOSL1 showed decreased migration and increased adhesion on vitronectin. At the morphological level, the focal adhesions and the actin stress fibers were not aligned in the direction of cell migration but randomly distributed. Focal adhesions are comprised of clusters of integrin receptors associated with large complexes of structural and signaling proteins linked to the actin cytoskeleton. Integrins in the focal adhesions have the structural function to connect actin stress fibers to the ECM and a signaling function to regulate the organization of the actin cytoskeleton as well. The constant remodeling of the actin stress fibers and the rapid assembly/disassembly of the focal adhesions are essential for endothelial cell migration, which requires continuous adhesion/de-adhesion processes. In this coordinated process, integrins must disengage from ECM contacts at the trailing end and form new focal contacts at the leading edge. Thus, dysregulation of $\alpha v\beta 3$ integrin complex in FOSL1-silenced cells results in the increased size and stabilization of focal adhesions with the consequent increased cell adhesion and inhibition of migration. This was confirmed by lentiviral vector-mediated over-expression of $\alpha v\beta 3$ integrin, that showed phenotypic alterations similar to those obtained by FOSL1 silencing, thus suggesting that the expression of FOSL1 controlling the level of $\alpha v\beta 3$ integrin complex is crucial for functional focal contacts, cytoskeleton organization, and migration.

FOSL1 is a basic-leucine zipper (bZIP) protein that must dimerize with proteins of the JUN family to form the active transcription factor complex AP-1. Using ChIP assay, we demonstrated that JUND binds to both αv and $\beta 3$ promoters in a FOSL1-dependent manner suggesting that JUND is the heterodimeric binding partner of FOSL1 on these promoters. However, no AP-1 binding sites are present in αv and $\beta 3$ promoters. Indeed, we found that FOSL1 binds on αv and $\beta 3$ promoters through its interaction with the nuclear factor SP1 (Figure 1), similarly to what happens with the v-Jun homodimer on the SPARC promoter.¹⁹ Interestingly, in both cases the indirect binding of an AP-1 factor through SP1 act as a repressor of the transcription.

Endothelial cells migration also requires ECM proteolysis and remodeling. Besides $\alpha v\beta 3$ integrin, we also identified *uPA* and *uPAR* as FOSL1-regulated genes in endothelial cells. In this case FOSL1 promotes their expression (Figure 2).¹⁶ The urokinase receptor (uPAR) is a specific receptor for the serine protease urokinase plasminogen activator (uPA). The uPA-uPAR complex is exposed at the cell surface and contributes to ECM remodelling by directing proteolytic activity at the leading edge of migrating cells.²⁰ In addition to its central role in ECM proteolysis, the uPA-uPAR complex acts as a co-receptor of integrins and is important for their activation and redistribution during focal adhesion turnover.^{21,22} In fact, uPA-uPAR complex has been shown to interact with vitronectin and is required to activate $\alpha v\beta 3$ integrin in podocytes, promoting cell migration.^{23,24}

Upon bFGF treatment, which promotes endothelial cells activation, endothelial cells respond with the induction of $\alpha v\beta 3$ complex and uPA,⁸ but also with the activation of FOSL1.²⁵ Our results are compatible with a model in which FOSL1 functions as a modulator of both complexes to precisely tuning the level of key molecules on endothelial cell surface. FOSL1 can function as either an activator or a repressor, depending on the gene-context, controlling in this way the delicate equilibrium between adhesion and migration in ongoing angiogenesis.

In conclusion, while more information is available about $\beta 3$ regulation, very little is known about transcription factors directly regulating αv integrin in any cell types.

Our group has demonstrated that FOSL1 is a direct down-modulator of both αv and $\beta 3$ integrin subunits in activated endothelial cells, but given that this complex is a worthy candidate for anti-angiogenic therapy in cancer, it will be interesting also to determine the transcription factor(s) that mediates the specific induction of the αv subunit.

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Legend to Figures

Figure 1. Schematic representation of FOSL1 binding and negative regulation of the α and β 3 promoters in endothelial cells. The heterodimer FOSL1/JUND binds promoters DNA through SP1 and recruits histone deacetylase complex to repress transcription. Green flags represent acetyl groups on H4K16.

Figure 2. Expression level of uPA and uPAR mRNAs in HUVEC silenced for FOSL1 and in FOSL1-silenced cells infected with a viral vector expressing an shRNA-resistant FOSL1 mutant (rescue). Results were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA. Mean values from three independent experiments are shown. Standard deviations are indicated.



